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Technical Report Series
No. 333

TOXICOLOGY AND CARCINOGENESIS STUDIES OF *N*-PHENYL-2-NAPHTHYLAMINE (CAS NO. 135-88-6) IN F344/N RATS AND B6C3F$_1$ MICE (FEED STUDIES)

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
Public Health Service
National Institutes of Health

COMPLETED

ORIGINAL

NATIONAL TOXICOLOGY PROGRAM

The National Toxicology Program (NTP), established in 1978, develops and evaluates scientific information about potentially toxic and hazardous chemicals. This knowledge can be used for protecting the health of the American people and for the primary prevention of disease. By bringing together the relevant programs, staff, and resources from the U.S. Public Health Service, DHHS, the National Toxicology Program has centralized and strengthened activities relating to toxicology research, testing and test development/validation efforts, and the dissemination of toxicological information to the public and scientific communities and to the research and regulatory agencies.

The NTP is made up of four charter DHHS agencies: the National Cancer Institute (NCI), National Institutes of Health; the National Institute of Environmental Health Sciences (NIEHS), National Institutes of Health; the National Center for Toxicological Research (NCTR), Food and Drug Administration; and the National Institute for Occupational Safety and Health (NIOSH), Centers for Disease Control. In July 1981, the Carcinogenesis Bioassay Testing Program, NCI, was transferred to the NIEHS.

NTP TECHNICAL REPORT

ON THE

TOXICOLOGY AND CARCINOGENESIS STUDIES OF *N*-PHENYL-2-NAPHTHYLAMINE

(CAS NO. 135-88-6)

IN F344/N RATS AND B6C3F$_1$ MICE

(FEED STUDIES)

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Public Health Service
National Institutes of Health

NOTE TO THE READER

This study was performed under the direction of the National Institute of Environmental Health Sciences as a function of the National Toxicology Program. The studies described in this Technical Report have been conducted in compliance with NTP chemical health and safety requirements and must meet or exceed all applicable Federal, state, and local health and safety regulations. Animal care and use were in accordance with the U.S. Public Health Service Policy on Humane Care and Use of Animals. All NTP toxicology and carcinogenesis studies are subjected to a data audit before being presented for public peer review.

Although every effort is made to prepare the Technical Reports as accurately as possible, mistakes may occur. Readers are requested to identify any mistakes so that corrective action may be taken. Further, anyone who is aware of related ongoing or published studies not mentioned in this report is encouraged to make this information known to the NTP. Comments and questions about the National Toxicology Program Technical Reports on Toxicology and Carcinogenesis Studies should be directed to Dr. J.E. Huff, National Toxicology Program, P.O. Box 12233, Research Triangle Park, NC 27709 (919-541-3780).

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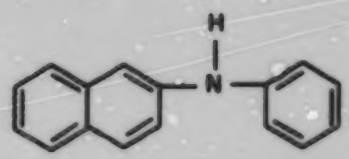

_N_-PHENYL-2-NAPHTHYLAMINE

CAS No. 135-88-6

$C_{16}H_{13}N$ Molecular weight 219.3

Synonyms: _N_-(2-naphthyl)aniline; 2-naphthylphenylamine; β-naphthylphenylamine; 2-phenylaminonaphthalene; phenyl-β-naphthylamine; _N_-phenyl-β-naphthylamine

Trade names: Aceto PBN; Agerite Powder: Antioxidant 116; Neozone D; Neozon D; Nilox PBNA; Nonox D; PBNA; Stabilizator AR

ABSTRACT

_N_-Phenyl-2-naphthylamine, formerly used as an antioxidant in the rubber industry, was selected for toxicology and carcinogenesis studies because at the time of nomination (1976) it had a large annual production and widespread human exposure. Additional reasons for selection included its structural similarity and possible metabolism to the known human urinary bladder carcinogen, 2-naphthylamine. Toxicology and carcinogenesis studies were conducted by feeding diets containing _N_-phenyl-2-naphthylamine (approximately 98% pure and containing less than 1 ppm 2-naphthylamine) at various concentrations to groups of F344/N rats and B6C3F$_1$ mice of each sex for 14 days, 13 weeks, or 2 years.

Fourteen-Day and Thirteen-Week Studies: **In 14-day studies, 3/5 male and 4/5 female rats that received 50,000 ppm _N_-phenyl-2-naphthylamine died before the end of the studies. Final mean body weights of rats that received 12,500 ppm or more were considerably lower (18%-57%) than those of the controls. Arched backs, rough coats, and diarrhea were observed for males that received 12,500 ppm or more and for females that received 25,000 or 50,000 ppm. All mice were alive at the end of the studies, and no compound-related clinical signs of toxicity were observed in mice given feed containing up to 20,000 ppm.**

In 13-week studies, deaths occurred in 4/10 male and 9/10 female rats that received the highest dose (40,000 ppm) of _N_-phenyl-2-naphthylamine. Final mean body weights of rats that received 5,000-40,000 ppm were 9%-60% lower than those of the controls. The liver weight to body weight ratios increased with increasing dose, with the ratios for male rats at 10,000 ppm or more and for female rats at 5,000 ppm being greater ($P<0.05$) than those of the controls. A compound-related nephropathy occurred in rats and was characterized by renal tubular epithelial degeneration and hyperplasia. Other effects in rats included hematopoietic hypoplasia or atrophy of the femoral bone marrow, testicular hypospermatogenesis, lymphoid degeneration of the thymus, and lymphoid depletion of the spleen.

In mice, 2/10 males and 7/10 females that received 40,000 ppm died before the end of the 13-week studies. The final mean body weights of mice that received 10,000, 20,000, or 40,000 ppm were 9%-32% lower than those of the controls. The liver weight to body weight ratios for mice increased with increasing dose. Those for male mice at 10,000 ppm or more and for female mice at 20,000 ppm or

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more were greater ($P<0.05$) than those for the controls. Nephropathy was observed at increased incidences and severity in dosed mice.

Because of kidney lesions, liver enlargement, lower weight gain, and increased mortality in the shorter term studies, dietary concentrations of *N*-phenyl-2-naphthylamine selected for the 2-year studies in rats and in mice were 0, 2,500, and 5,000 ppm.

Body Weight and Survival in the Two-Year Studies: The mean body weights of dosed rats were lower than those of the controls throughout the studies (12% and 16% lower for dosed males and 15% and 31% lower for dosed females at the end of the studies). The average daily feed consumption for rats was 94%-97% that of the controls for dosed males and 88% that of the controls for dosed females. The estimated average amount of *N*-phenyl-2-naphthylamine consumed per day was 100 mg/kg and 225 mg/kg for male rats and 120 mg/kg and 260 mg/kg for female rats. The survival of the high dose group of male rats was greater ($P<0.05$) than that of the controls after week 101 (male: control, 24/50; low dose, 28/50; high dose, 34/50; female: 36/50; 44/50; 38/50).

Final mean body weights of high dose male and female mice were lower (male, 9%; female, 23%) than those of the controls. The estimated average daily feed consumption by dosed mice was within 10% that of the controls. The average amount of *N*-phenyl-2-naphthylamine consumed per day was approximately 500 or 1,000 mg/kg for male mice and 450 or 900 mg/kg for female mice. No significant differences in survival were observed between any groups of mice of either sex (male: control, 33/50; low dose, 36/50; high dose, 28/50; female: 36/50; 30/50; 35/50).

Nonneoplastic and Neoplastic Effects in the Two-Year Studies: As in the 13-week studies, the kidney was the principal target for the toxic effects of *N*-phenyl-2-naphthylamine. Mineralization of the kidney, necrosis of the renal papilla, and epithelial hyperplasia and calculi of the kidney pelvis were observed at increased incidences in dosed female rats. Hydronephrosis, atrophy, fibrosis, and chronic focal inflammation of the kidney were observed at increased incidences in high dose female rats. Cysts and acute suppurative inflammation of the kidney were observed at increased incidences in dosed male and high dose female rats. No compound-related renal neoplasms were observed in rats.

Nuclear enlargement of renal tubular epithelial cells and nephropathy were observed at increased incidences in high dose female mice. Atypical tubular cell hyperplasia occurred in two high dose female mice. A tubular cell adenoma was found in one high dose female mouse, and a tubular cell adenocarcinoma was found in another high dose female mouse. No renal neoplasms were observed in dosed male mice.

Neoplasms of several organs occurred in rats with negative trends and/or at significantly lower incidences in high dose groups. These included thyroid gland C-cell neoplasms in males and females and mammary gland fibroadenomas, pituitary gland adenomas, and mononuclear cell leukemia in females. The lack of carcinogenicity in rats may be related to an inability to metabolize this compound to the known animal and human carcinogen 2-napthylamine.

Genetic Toxicology: N-Phenyl-2-naphthylamine was not mutagenic in the *Salmonella typhimurium*/microsome assay with strains TA97, TA98, TA100, or TA1535 with or without induced hamster or rat liver S9. The chemical did not induce chromosomal aberrations in cultured Chinese hamster ovary (CHO) cells with or without metabolic activation. No increase in sister chromatid exchanges (SCEs) was observed in the absence of metabolic activation; in the presence of rat liver S9, the SCE results were judged to be equivocal.

Data Audit: The data, documents, and pathology materials from the 2-year studies of *N*-phenyl-2-naphthylamine were audited at the NTP Archives. The audit findings show that the conduct of the studies is documented adequately and support the data and results given in this Technical Report.

Conclusions: Under the conditions of these 2-year feed studies, there was *no evidence of carcinogenic activity** for male or female F344/N rats fed diets containing 2,500 or 5,000 ppm *N*-phenyl-2-naphthylamine. Decreased incidences of several neoplasms were observed in dosed rats: thyroid gland C-cell neoplasms in males and females and mononuclear cell leukemia, pituitary gland adenomas, and mammary gland fibroadenomas in females. There was *no evidence of carcinogenic activity* for male B6C3F$_1$ mice fed diets containing 2,500 or 5,000 ppm *N*-phenyl-2-naphthylamine. There was *equivocal evidence of carcinogenic activity* of *N*-phenyl-2-naphthylamine for female B6C3F$_1$ mice as indicated by the occurrence of two rare kidney neoplasms. Chemical-related nonneoplastic lesions (nephropathy, karyomegaly, and hyperplasia) occurred in the kidney of rats and mice.

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*Explanation of Levels of Evidence of Carcinogenic Activity is on page 7.
A summary of the Peer Review comments and the public discussion on this Technical Report appears on page 11.

SUMMARY OF THE NTP TWO-YEAR FEED STUDIES, GENETIC TOXICOLOGY, AND METABOLISM OF *N*-PHENYL-2-NAPHTHYLAMINE

Male F344/N Rats	Female F344/N Rats	Male B6C3F$_1$ Mice	Female B6C3F$_1$ Mice
Dietary concentration			
0, 2,500, or 5,000 ppm *N*-phenyl-2-naphthylamine	0, 2,500, or 5,000 ppm *N*-phenyl-2-naphthylamine	0, 2,500, or 5,000 ppm *N*-phenyl-2-naphthylamine	0, 2,500, or 5,000 ppm *N*-phenyl-2-naphthylamine
Survival rates in the 2-year studies			
34/50; 21/50; 24/50	39/50; 44/50; 38/50	33/50; 36/50; 33/50	38/50; 20/50; 35/50
Nonneoplastic effects			
Kidney: cysts, chronic focal and acute suppurative inflammation of tubules	Kidney: cysts, chronic focal and acute suppurative inflammation of tubules, mineralization, necrosis, calculi, hyperplasia, hydronephrosis, atrophy, fibrosis	None	Kidney: karyomegaly, nephropathy
Neoplastic effects			
Decrease in incidence of thyroid gland C-cell adenomas or carcinomas (combined)	Decrease in incidences of thyroid gland C-cell adenomas, carcinomas, and adenomas or carcinomas (combined); mammary gland fibroadenomas; pituitary gland adenomas; mononuclear cell leukemia	None	Increase in incidences of renal tubular cell adenomas and tubular cell adenocarcinomas
Level of evidence of carcinogenic activity			
No evidence	No evidence	No evidence	Equivocal evidence
Other considerations			
Increase in relative liver weights at 10,000 ppm or more in the 13-wk study	Increase in relative liver weights at 5,000 ppm or more in the 13-wk study	Increase in relative liver weights at 10,000 ppm or more in the 13-wk study	Increase in relative liver weights at 20,000 ppm or more in the 13-wk study

Genetic toxicology
Not mutagenic in *S. typhimurium* strains TA97, TA98, TA100, or TA1535 with or without metabolic activation; did not induce chromosomal aberrations in CHO cells with or without metabolic activation or SCEs without metabolic activation; results of SCE test in the presence of metabolic activation were equivocal.

Metabolism
Not metabolized to 2-naphthylamine in male F344/N rats

EXPLANATION OF LEVELS OF EVIDENCE OF CARCINOGENIC ACTIVITY

These studies are designed and conducted to characterize and evaluate the toxicologic potential, including carcinogenic activity, of selected chemicals in laboratory animals (usually two species, rats and mice). Chemicals selected for NTP toxicology and carcinogenesis studies are chosen primarily on the bases of human exposure, level of production, and chemical structure. Selection per se is not an indicator of a chemical's carcinogenic potential.

Negative results, in which the study animals do not have a greater incidence of neoplasia than control animals, do not necessarily mean that a chemical is not a carcinogen, inasmuch as the experiments are conducted under a limited set of conditions. Positive results demonstrate that a chemical is carcinogenic for laboratory animals under the conditions of the study and indicate that exposure to the chemical has the potential for hazard to humans.

The National Toxicology Program describes the results of individual experiments on a chemical agent and notes the strength of the evidence for conclusions regarding each study. Other organizations, such as the International Agency for Research on Cancer, assign a strength of evidence for conclusions based on an examination of all available evidence including: animal studies such as those conducted by the NTP, epidemiologic studies, and estimates of exposure. Thus, the actual determination of risk to humans from chemicals found to be carcinogenic in laboratory animals requires a wider analysis that extends beyond the purview of these studies.

Five categories of evidence of carcinogenic activity are used in the Technical Report series to summarize the strength of the evidence observed in each experiment: two categories for positive results ("Clear Evidence" and "Some Evidence"); one category for uncertain findings ("Equivocal Evidence"); one category for no observable effects ("No Evidence"); and one category for experiments that because of major flaws cannot be evaluated ("Inadequate Study"). These categories of interpretative conclusions were first adopted in June 1983 and then revised in March 1986 for use in the Technical Reports series to incorporate more specifically the concept of actual weight of evidence of carcinogenic activity. For each separate experiment (male rats, female rats, male mice, female mice), one of the following quintet is selected to describe the findings. These categories refer to the strength of the experimental evidence and not to either potency or mechanism.

- **Clear Evidence of Carcinogenic Activity** is demonstrated by studies that are interpreted as showing a dose-related (i) increase of malignant neoplasms, (ii) increase of a combination of malignant and benign neoplasms, or (iii) marked increase of benign neoplasms if there is an indication from this or other studies of the ability of such tumors to progress to malignancy.
- **Some Evidence of Carcinogenic Activity** is demonstrated by studies that are interpreted as showing a chemically related increased incidence of neoplasms (malignant, benign, or combined) in which the strength of the response is less than that required for clear evidence.
- **Equivocal Evidence of Carcinogenic Activity** is demonstrated by studies that are interpreted as showing a marginal increase of neoplasms that may be chemically related.
- **No Evidence of Carcinogenic Activity** is demonstrated by studies that are interpreted as showing no chemically related increases in malignant or benign neoplasms.
- **Inadequate Study of Carcinogenic Activity** is demonstrated by studies that because of major qualitative or quantitative limitations cannot be interpreted as valid for showing either the presence or absence of carcinogenic activity.

When a conclusion statement for a particular experiment is selected, consideration must be given to key factors that would extend the actual boundary of an individual category of evidence. This should allow for incorporation of scientific experience and current understanding of long-term carcinogenesis studies in laboratory animals, especially for those evaluations that may be on the borderline between two adjacent levels. These considerations should include:

- The adequacy of the experimental design and conduct;
- Occurrence of common versus uncommon neoplasia;
- Progression (or lack thereof) from benign to malignant neoplasia as well as from preneoplastic lesions;
- Some benign neoplasms have the capacity to regress but others (of the same morphologic type) progress. At present, it is impossible to identify the difference. Therefore, where progression is known to be a possibility, the most prudent course is to assume that benign neoplasms of those types have the potential to become malignant;
- Combining benign and malignant tumor incidences known or thought to represent stages of progression in the same organ or tissue;
- Latency in tumor induction;
- Multiplicity in site-specific neoplasia;
- Metastases;
- Supporting information from proliferative lesions (hyperplasia) in the same site of neoplasia or in other experiments (same lesion in another sex or species);
- The presence or absence of dose relationships;
- The statistical significance of the observed tumor increase;
- The concurrent control tumor incidence as well as the historical control rate and variability for a specific neoplasm;
- Survival-adjusted analyses and false positive or false negative concerns;
- Structure-activity correlations; and
- In some cases, genetic toxicology.

These considerations together with the definitions as written should be used as composite guidelines for selecting one of the five categories. Additionally, the following concepts (as patterned from the International Agency for Research on Cancer Monographs) have been adopted by the NTP to give further clarification of these issues:

> The term *chemical carcinogenesis* generally means the induction by chemicals of neoplasms not usually observed, the induction by chemicals of more neoplasms than are generally found, or the earlier induction by chemicals of neoplasms that are commonly observed. Different mechanisms may be involved in these situations. Etymologically, the term *carcinogenesis* means induction of cancer, that is, of malignant neoplasms; however, the commonly accepted meaning is the induction of various types of neoplasms or of a combination of malignant and benign neoplasms. In the Technical Reports, the words *tumor* and *neoplasm* are used interchangeably.

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PEER REVIEW PANEL

The members of the Peer Review Panel who evaluated the draft Technical Report on *N*-phenyl-2-naphthylamine on March 4, 1987, are listed below. Panel members serve as independent scientists, not as representatives of any institution, company, or governmental agency. In this capacity, Panel members have five major responsibilities: (a) to ascertain that all relevant literature data have been adequately cited and interpreted, (b) to determine if the design and conditions of the NTP studies were appropriate, (c) to ensure that the Technical Report presents the experimental results and conclusions fully and clearly, (d) to judge the significance of the experimental results by scientific criteria, and (e) to assess the evaluation of the evidence of carcinogenicity and other observed toxic responses.

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SUMMARY OF PEER REVIEW COMMENTS ON THE TOXICOLOGY AND CARCINOGENESIS STUDIES OF *N*-PHENYL-2-NAPHTHYLAMINE

On March 4, 1987, the draft Technical Report on the toxicology and carcinogenesis studies of *N*-phenyl-2-naphthylamine received peer review by the National Toxicology Program Board of Scientific Counselors' Technical Reports Review Subcommittee and associated Panel of Experts. The review meeting was held at the National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina.

Dr. K. Abdo, NTP, introduced the toxicology and carcinogenesis studies of *N*-phenyl-2-naphthylamine in rats and mice by reviewing the experimental design, results, and proposed conclusions (no evidence of carcinogenic activity for male or female rats or for male mice, equivocal evidence of carcinogenic activity for female mice).

Dr. Sivak, a principal reviewer, agreed with the conclusions for male and female rats and male mice. He proposed that the conclusion for female mice be changed to no evidence of carcinogenic activity, saying that the presence of only one benign and one malignant renal tumor and the absence of any genotoxic response made this designation more appropriate.

As a second principal reviewer, Dr. Capen agreed with the conclusions for male and female rats and male mice while giving support to changing the conclusion for female mice to no evidence of carcinogenic activity.

Dr. Perera, a third principal reviewer, was unable to attend the meeting; her written comments were read by Dr. L. Hart, NIEHS. Dr. Perera agreed with the conclusions for female rats and male and female mice but thought that the conclusion for male rats should be changed to equivocal evidence of carcinogenic activity, based on the increased incidence of rare tumors of the spleen and two rare tumors of the colon. She said that the supporting evidence for the conclusion in female mice should be expanded to include "...as well as karyomegaly of tubular epithelial cells and atypical cell hyperplasia."

In response to Dr. Sivak and Dr. Capen, Dr. Abdo explained that the conclusion of equivocal evidence of carcinogenic activity in female mice was made because the kidney is a target organ for the chemical, the incidence of kidney tumors in the high dose group was 4% whereas the historical incidence at the study laboratory is 0%, and atypical hyperplasia was present. Dr. Sivak agreed that with mention of the nonneoplastic lesions he could support the original conclusions. He said that justification for the conclusion in female mice should cite not only the kidney neoplasms but also the occurrence of hyperplasia and nuclear enlargement as well as enhanced nephropathy in the high dose group. Dr. Abdo also explained that the conclusion chosen for male rats was appropriate because splenic tumors are not as rare as previously thought, whereas the colon tumors are mesenchymal rather than epithelial in origin and there is no evidence to suggest that the colon is a target organ.

Dr. Sivak moved that the Technical Report on *N*-phenyl-2-naphthylamine be accepted with the revisions discussed and the conclusions as written for male and female rats and male mice, no evidence of carcinogenic activity, and for female mice, equivocal evidence of carcinogenic activity. Dr. Capen seconded the motion, and it was approved unanimously with seven votes.

CONTRIBUTORS

The NTP Technical Report on the Toxicology and Carcinogenesis Studies of *N*-Phenyl-2-naphthylamine is based on the 13-week studies that began in June 1980 and ended in September 1980 and on the 2-year studies that began in April 1981 and ended in May 1983 at Battelle Columbus Laboratories.

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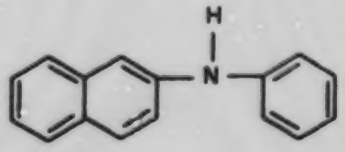
I. INTRODUCTION

Physical and Chemical Properties
Production and Use
Environmental Occurrence and Human Exposure
Toxicity
Evidence of Carcinogenic Activity for Humans
Evidence of Carcinogenic Activity for Animals
Metabolism
Genetic Toxicology
Study Rationale

I. INTRODUCTION



***N*-PHENYL-2-NAPHTHYLAMINE**

CAS No. 135-88-6

$C_{16}H_{13}N$ Molecular weight 219.3

Synonyms: *N*-(2-naphthyl)aniline; 2-naphthylphenylamine; β-naphthylphenylamine; 2-phenylaminonaphthalene; phenyl-β-naphthylamine; *N*-phenyl-β-naphthylamine

Trade names: Aceto PBN; Agerite Powder: Antioxidant 116; Neosone D; Neozon D; Nilox PBNA; Nonox D; PBNA; Stabilizator AR

***N*-Phenyl-2-naphthylamine is a synthetic antioxidant formerly used primarily in the processing of rubber. The technical product is at least 97% pure with a maximum of 0.5% ash and 0.5% 2-naphthol. *N*-Phenyl-2-naphthylamine was first prepared in 1880 by Graebe by heating 2-naphthol with aniline in the presence of a catalyst (IARC, 1978).**

Physical and Chemical Properties

Pure *N*-phenyl-2-naphthylamine occurs as gray to tan flakes or powder, forming rhombic crystals when recrystallized from methyl alcohol. It has a melting point of 107°-108° C and a boiling point of 395° C. It is insoluble in water but soluble in ethyl alcohol (50 g/liter), benzene (27 g/liter), and acetone (640 g/liter) (Sax, 1984; IARC, 1978).

Production and Use

U.S. production of *N*-phenyl-2-naphthylamine was 2.05 million kg in 1972, 2.24 million kg in 1973, 1.37 million kg in 1974, and 709,000 kg in 1975 (USITC, 1975, 1976, 1977). Current information indicates that *N*-phenyl-2-naphthylamine is no longer produced or used in the United States, possibly because studies in humans indicated that this compound is partially metabolized to 2-naphthylamine, a known human carcinogen (Moore et al., 1977). U.S. Department of Commerce data for imports and exports during 1985 do not indicate any trade activity for this compound. *N*-Phenyl-2-naphthylamine was used primarily as an antioxidant in rubber processing at levels ranging from 1% to 2% to increase resistance of rubber to heat, oxidation, and cracking (Kehe and Kouris, 1965; IARC, 1978). It has been used as an antioxidant in grease and oils and as a stabilizer in the manufacture of dyes and silicone enamels (Kehe and Kouris, 1965).

Environmental Occurrence and Human Exposure

According to an EPA study titled "Frequency of Organic Compounds Identified in Water," a compound identified only as "phenylnaphthylamine" was reported to have been detected in water at two geographic locations (NCI, 1977). Approximately 15,000 rubber workers were exposed to *N*-phenyl-2-naphthylamine in 1977 (NIOSH, 1977). Because of the finding that humans metabolize *N*-phenyl-2-naphthylamine to 2-naphthylamine, the National Institute for Occupational Safety and Health made recommendations and suggested a number of industrial hygiene guidelines to minimize exposure (NIOSH, 1977).

Toxicity

Little is known about the toxicity of *N*-phenyl-2-naphthylamine. The reported oral LD_{50} values are 8,730 mg/kg for rats and 1,450 mg/kg for mice (Sax, 1984). Acute vascular changes in the

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liver, lung, and brain as a result of venous congestion were observed in rats at a dose equal to the LD_{50} value. Gavage administration of 1,750 mg/kg (20% of the LD_{50} value) to rats for 1 month caused clinical signs of lethargy, somnolence, diminished appetite, and some reduction in weight.

Evidence of Carcinogenic Activity for Humans

An epidemiologic study of workers who entered the rubber industry after 1949 (when *N*-phenyl-2-naphthylamine replaced 2-naphthylamine) indicated that the risk of cancer for workers was not significantly greater than that for the general population, but the authors considered their data to be inconclusive (Fox and Collier, 1976). A more recent epidemiologic study of rubber workers in Shanghai reported an excessive incidence of lung cancer in those involved in compounding, mixing, and milling (Wang et al., 1984a). The higher incidence of lung cancer in rubber industry workers relative to that in workers in other industries was considered to be associated with high levels of *N*-phenyl-2-naphthylamine in the atmosphere.

Evidence of Carcinogenic Activity for Animals

Groups of 18 male and 18 female (C57BL/6 × C3H/Anf)F_1 mice and a similar number of male and female (C57BL/6 × AKR)F_1 mice were given 464 mg *N*-phenyl-2-naphthylamine/kg body weight per day by gavage in aqueous gelatin from 7 to 28 days of age and then 1,206 ppm in feed until the mice were killed at 78 weeks of age (Innes et al., 1969). A significantly increased incidence of neoplasms was observed in males of the first strain, mainly because of the increase in the incidence of hepatomas. In a similar experiment in which the same number of 28-day-old mice of each sex and strain were given a single subcutaneous injection of 464 mg *N*-phenyl-2-naphthylamine/kg body weight in dimethyl sulfoxide (DMSO) and observed up to 80 weeks of age, there were significant increases in the number of females of the first strain with tumors and in the number of males of the second strain with hepatomas (Innes et al., 1969; IARC, 1978).

Male ICR mice given subcutaneous injections of 16 mg technical-grade *N*-phenyl-2-naphthylamine in 0.1 ml DMSO three times per week for 9 weeks and observed for an additional 32 weeks had a higher incidence of malignant tumors relative to that of DMSO vehicle controls (malignant tumors: 0/24 vs. 9/26; lung carcinomas: 0/24 vs. 6/26; kidney carcinomas: 0/24 vs. 1/26) (Wang et al., 1984b). In a similar study with unilaterally nephrectomized male TA-1 mice, subcutaneous injections of 16 mg pure *N*-phenyl-2-naphthylamine per mouse for a total dose of 328 mg over 273 days resulted in a significant increase in the number of animals with malignant tumors (malignant tumors: 0/18 in intact controls vs. 12/16; kidney hemangiosarcomas: 0/18 vs. 12/16).

No evidence of tumorigenic activity was observed in Syrian golden hamsters given 37.5 or 75 mg *N*-phenyl-2-naphthylamine/kg body weight intragastrically twice a week for life (Green et al., 1979). No urinary bladder tumors were observed in three dogs fed 540 mg *N*-phenyl-2-naphthylamine 5 days a week for 4.5 years (Gehrmann et al., 1949). Sprague Dawley rats (40 males and 40 females) given 600 mg *N*-phenyl-2-naphthylamine/kg body weight in 0.5 ml arachidis oil by gavage two times per week for life showed no evidence of compound-related effects on survival, incidence of tumors, tumor latency, or tumor multiplicity (Ketkar and Mohr, 1982).

Metabolism

Dephenylation of *N*-phenyl-2-naphthylamine to 2-naphthylamine has been reported to occur in rats and dogs, as well as in humans. Volunteers given a single oral dose (10 or 20 mg) of technical-grade *N*-phenyl-2-naphthylamine excreted 2-naphthylamine in urine (Kummer and Tordoir, 1975). Conversion of *N*-phenyl-2-naphthylamine to 2-naphthylamine in humans was confirmed in studies conducted by the B.F. Goodrich Company (NIOSH, 1977). In these studies, 3-4 mg of 2-naphthylamine was found in 24-hour urine samples from two volunteers who ingested 50 mg *N*-phenyl-2-naphthylamine (containing 0.7 µg 2-naphthylamine) and from workers who inhaled an estimated 30 mg *N*-phenyl-2-naphthylamine. Beagle dogs fed a

single dose of the chemical at 5 mg/kg body weight excreted up to 10 µg 2-naphthylamine in urine (Batten and Hathway, 1977). Sprague Dawley rats given a single dose of 50 mg per day in 0.5 ml aqueous gelatin or 100 mg per day in 1 ml aqueous gelatin for 4 days excreted the parent compound (20 µg and 840 µg) and 2-naphthylamine (1.4 µg and 34 µg) in urine (Laham and Potvin, 1983). The authors concluded that *N*-phenyl-2-naphthylamine enhances its own metabolism on repeated dosing because of the eightfold difference in the 2-naphthylamine concentration in urine in the day-4 samples as compared with that in the day-1 samples; no renal toxicity was reported in the study. *N*-Phenyl-2-naphthylamine and traces of 2-naphthylamine (less than 1 ppm) were found in the urine of male F344 rats fed diets containing 2,500 ppm or 5,000 ppm *N*-phenyl-2-naphthylamine for 7 days (SoRI, 1986). Only male rats were used in this study.

N-Phenyl-2-naphthylamine is metabolized by hepatic microsomal preparations from hamsters, rats, monkeys, dogs, and humans by the cytochrome P-450 mixed function oxidase system to 6-hydroxy-*N*-phenyl-2-naphthylamine and 4'-hydroxy-*N*-phenyl-2-naphthylamine; 2-naphthylamine was not detected (Anderson et al., 1982).

Genetic Toxicology

Results from short-term genotoxicity assays with *N*-phenyl-2-naphthylamine as reported in the literature and the data from the NTP studies are in general agreement, indicating that the compound is not mutagenic in either the presence or absence of exogenous metabolic activation. No increase in histidine revertant colonies was observed in experiments with *Salmonella typhimurium* strains TA98 or TA1535 incubated with *N*-phenyl-2-naphthylamine in the presence of induced mouse or hamster liver S9 in a plate incorporation assay with doses up to 2.7 µmol/plate (Bartsch et al., 1980) or with *S. typhimurium* strains TA98, TA100, TA1535, or TA1538 at doses up to 2,500 µg/plate in the presence of S9 (Anderson and Styles, 1978). The NTP *S. typhimurium*/microsome assays demonstrated that *N*-phenyl-2-naphthylamine was not mutagenic to strains TA97, TA98, TA100, or TA1535 when tested by a preincubation protocol at doses up to 333 µg/plate with or without Aroclor 1254-induced male Sprague Dawley rat or Syrian hamster liver S9 (Appendix E, Table E1). A review of the short-term test data generated from 1973 to 1978 in Japan, although providing no experimental details or references to original publications, described *N*-phenyl-2-naphthylamine as not mutagenic to *S. typhimurium* strains TA98 or TA100 in the presence of S9, not active in the *Bacillus subtilis* rec assay with or without metabolic activation, and not mutagenic to silkworms; in addition, it did not induce chromosomal aberrations in hamster lung fibroblast cells in vitro or rat bone marrow cells in vivo (Kawachi et al., 1980a,b). NTP in vitro cytogenetic assays with Chinese hamster ovary (CHO) cells demonstrated no induction of chromosomal aberrations with or without Aroclor 1254-induced male Sprague Dawley rat liver S9 after exposure to *N*-phenyl-2-naphthylamine at concentrations up to 29.7 µg/ml (Table E3). In the absence of rat liver S9, CHO cells demonstrated no increase in sister chromatid exchanges (SCEs) after incubation with *N*-phenyl-2-naphthylamine. In the presence of metabolic activation, an increase in SCEs was observed at the highest dose tested in each of two trials (Table E2); these results were judged to be equivocal because this increase was small relative to the baseline frequency in one trial and because there was no dose response but some toxicity in the other trial.

Of the several structural analogs of *N*-phenyl-2-naphthylamine, mutagenicity data are available on only one, *N*-phenyl-1-naphthylamine. This chemical was not mutagenic in in vitro assays with Salmonella, *Escherichia coli*, yeast, or cultured L5178Y mouse lymphoma cells in either the presence or absence of metabolic activation, nor did it induce dominant lethal mutations in germ cells of male mice given intraperitoneal injections of 500 mg/kg for 5 days (Brusick and Matheson, 1976). However, exposure to *N*-phenyl-1-naphthylamine did induce a slight, reproducible increase in unscheduled DNA synthesis in human WI-38 cells at one of the doses tested in the absence of metabolic activation, but there was no evidence of a dose-related trend. *N*-Phenyl-1-naphthylamine was also reported as not mutagenic in *S. typhimurium* strains TA98,

TA100, TA1535, or TA1537 with or without S9 in investigations conducted by Braden et al. (1978) and by the NTP (unpublished results). Results of NTP tests on *N*-phenyl-1-naphthylamine with cultured mammalian cells to detect chromosomal aberrations were also negative, but SCE rates were significantly increased after incubation of cells with *N*-phenyl-1-naphthylamine in the presence of rat liver S9.

2-Naphthylamine is a carcinogen that demonstrates mutagenic activity, especially in the presence of rat liver enzymes, in a wide range of in vitro (Dunkel et al., 1984; Gupta and Goldstein, 1981; Althaus et al., 1982; Wang et al., 1981; Natarajan and Van Kesteren-Van Leeuwen, 1981) and in vivo assays (Vogel et al., 1983; Sharma et al., 1980; Kirkhart, 1981; Parodi et al., 1983). It is a metabolite of *N*-phenyl-2-naphthylamine in dogs (Batten and Hathway, 1977) and humans (Moore et al., 1977).

Study Rationale

N-Phenyl-2-naphthylamine was nominated for toxicology and carcinogenesis studies by the National Cancer Institute because at the time of nomination it had a large annual production volume and widespread human exposure, structural similarity and possible metabolism to the known human urinary bladder carcinogen 2-naphthylamine (IARC, 1974), and lack of adequate data for evaluation of carcinogenicity. *N*-Phenyl-2-naphthylamine was administered in the diet because it is stable in feed and dietary administration was the most practical route of exposure.

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II. MATERIALS AND METHODS

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II. MATERIALS AND METHODS

PROCUREMENT AND CHARACTERIZATION OF *N*-PHENYL-2-NAPHTHYLAMINE

N-Phenyl-2-naphthylamine was obtained in one lot (lot no. 681) from Vulnax International, Ltd. (Chesford Grange, Woolston, United Kingdom). Purity and identity analyses were conducted at Midwest Research Institute (MRI) (Kansas City, Missouri). MRI reports on the analyses performed in support of the *N*-phenyl-2-naphthylamine studies are on file at NIEHS.

Lot no. 681 was obtained as a grey, microcrystalline powder with a melting point of 103°-108° C. The identity of *N*-phenyl-2-naphthylamine was confirmed by spectroscopic analysis. The infrared (Figure 1), ultraviolet/visible, and nuclear magnetic resonance (Figure 2) spectra were consistent with the literature spectra. The purity of *N*-phenyl-2-naphthylamine was determined by elemental analysis, Karl Fischer water analysis, titration of the amine group, thin-layer chromatography, and gas chromatography. The cumulative data indicated that *N*-phenyl-2-naphthylamine was approximately 98% pure. Results of elemental analyses agreed with the theoretical values for hydrogen and nitrogen and were slightly low for carbon. Water content was 0.26%. Titration of the amine group with 0.09 N perchloric acid indicated a purity of 97.9%. Thin-layer chromatography on Whatman KC_{18} reversed-phase plates with an acetonitrile:water (80:20) mobile phase indicated a major spot and three minor, two trace, and two slight trace impurities by ultraviolet light (254 and 366 nm), iodine vapor, and ninhydrin spray. Thin-layer chromatography on silica gel plates with a carbon tetrachloride:methanol (90:10) mobile phase indicated a major spot, five trace impurities, and one slight trace impurity by the same visualization methods. Gas chromatography with a 3% SP2100-DB column and flame ionization detection indicated a major peak and seven impurities with peak areas totaling 1.94% of the major peak area; one impurity had an area 1.3% of the major peak area. Gas chromatography with a 3% SP2401 column and flame ionization detection indicated a major peak and eight impurities with peak areas totaling 2.09% of the major peak area; three impurities had relative areas of 1.0%, 0.65%, and 0.27%. The major impurity present in the study material was identified by high-resolution gas chromatography/mass spectrometry as *N*-phenylphthalimide and was estimated to be present at a concentration of 1.3%.

High resolution gas chromatography/mass spectrometry analyses revealed the presence of four impurities with a peak area greater than 0.1%. Only one of these impurities was present at greater than 1%. This impurity was identified as 2-phenyl-1*H*-isoindole-1,3(2*H*)-dione.

The study material was examined for the presence of 2-naphthylamine. 2-Naphthylamine was extracted with 0.2 M hydrochloric acid from *N*-phenyl-2-naphthylamine in toluene. After neutralization and ether extraction, the *N*-naphthyl trifluoroacetamide derivative was prepared with trifluoroacetic anhydride and quantitated by gas chromatography with a 10% SP2100 column and flame ionization detection. 2-Naphthylamine was not present at the detection level of 1 ppm.

Stability studies performed by gas chromatography with a 3% SP2401 column indicated that *N*-phenyl-2-naphthylamine was stable in the dark at temperatures up to 60° C for at least 2 weeks. Further confirmation of the stability of the bulk chemical during the toxicity studies (storage at room temperature) was obtained by gas chromatography with a 3% SP2100-DB column and high-performance liquid chromatography on a μBondapak C_{18} column with a mobile phase of 1% acetic acid in water:1% acetic acid in acetonitrile (25:75) at a flow rate of 1 ml/minute and ultraviolet detection at 254 nm. No degradation was seen over the course of the studies. Identity of the chemical at the study laboratory was confirmed by infrared spectroscopy.

PREPARATION AND CHARACTERIZATION OF FORMULATED DIETS

Formulated diets were prepared by adding a dry premix of feed and *N*- phenyl-2-naphthylamine to the appropriate amount of feed and blending

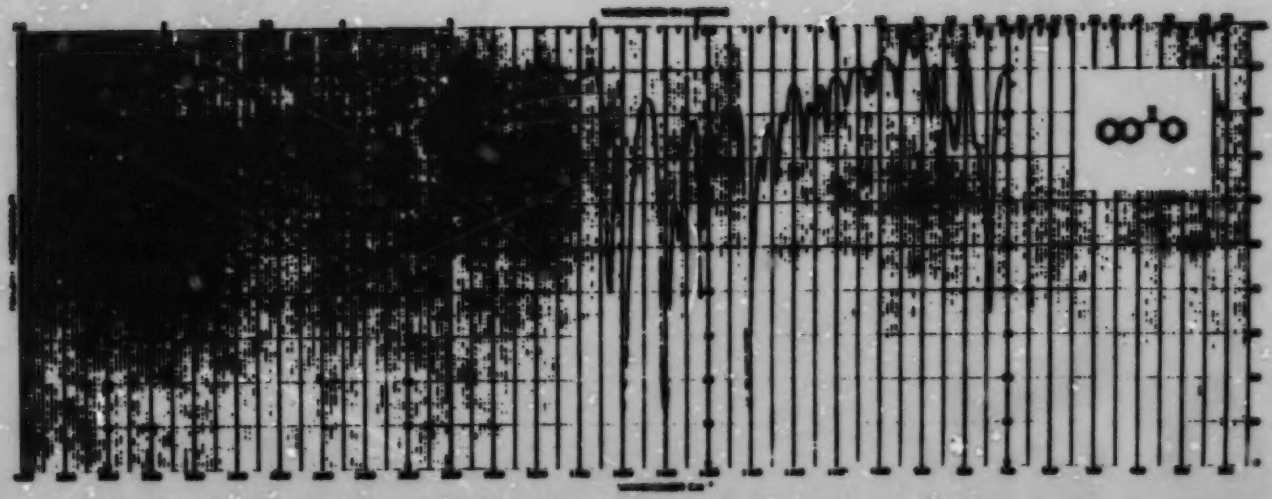

FIGURE 1. INFRARED ABSORPTION SPECTRUM OF *N*-PHENYL-2-NAPHTHYLAMINE (LOT NO. 681)

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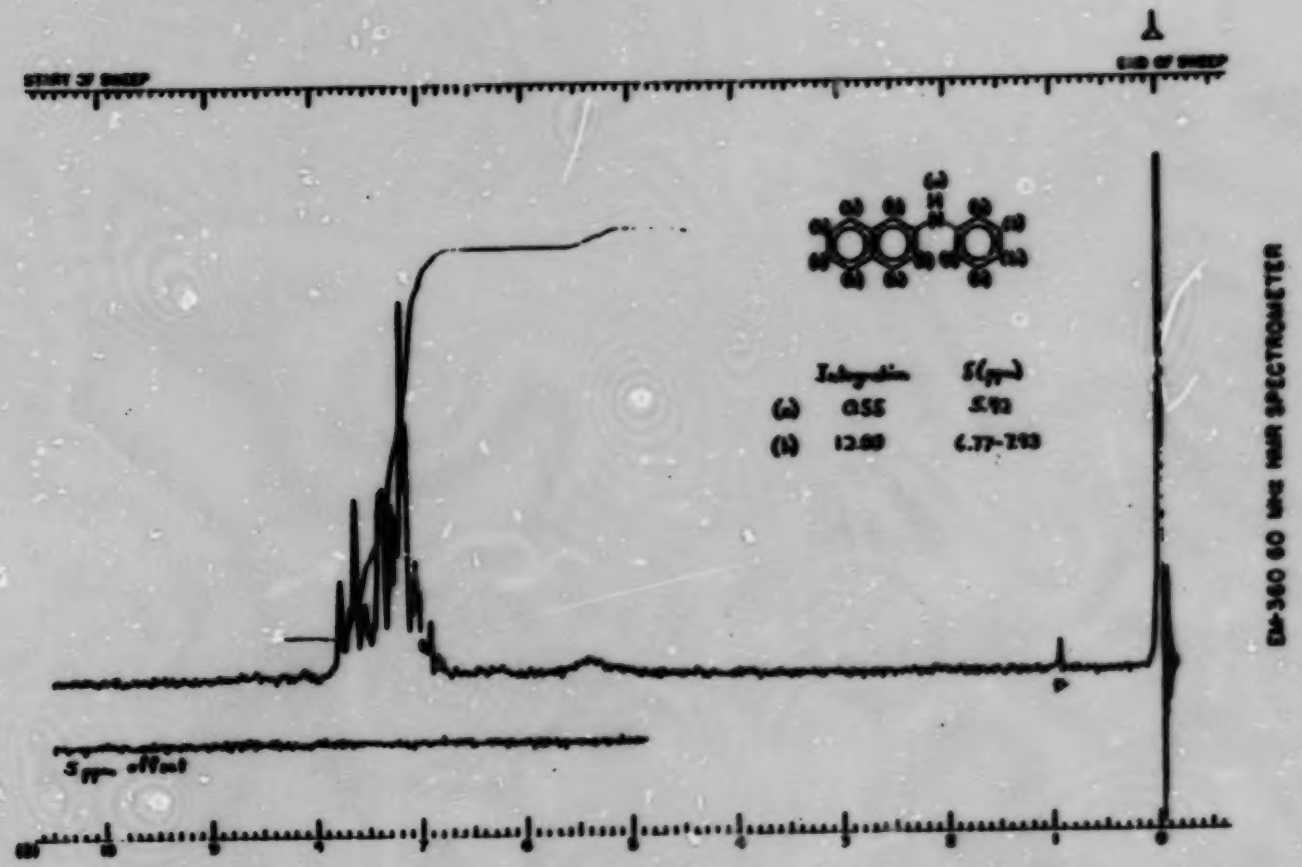

FIGURE 2. NUCLEAR MAGNETIC RESONANCE SPECTRUM OF *N*-PHENYL-2-NAPHTHYLAMINE (LOT NO. 881)

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for 15 minutes (Table 1). The homogeneity of formulated diets prepared at the analytical chemistry and study laboratories was evaluated by extracting feed samples (taken from three points of the blender) with a solution of acetonitrile:acetic acid (99:1) and determining the absorption at 271 nm. Spiked feed mixtures were analyzed in tandem to develop a standard curve. Good homogeneity was found in formulated diets prepared at both laboratories. At the analytical chemistry laboratory, less than 1% deviation from the target value was observed at a concentration of 5,000 ppm. At the study laboratory, values ranged from 92.3% to 95.0% of the target value at a concentration of 40,000 ppm and 94.0% to 99.6% at a concentration of 2,500 ppm. Further studies by high-performance liquid chromatography (with the same analytical parameters as those described above and the same acetonitrile:acetic acid [99:1] extraction step) showed that *N*-phenyl-2-naphthylamine at 5,000 ppm was stable in feed when stored in the dark for 2 weeks at 5° C. A loss of approximately 3% was demonstrated after 2 weeks' storage at 25° C.

High-performance liquid chromatography as described above but with a 60:40 solvent ratio at a flow rate of 2 ml/minute and fluorescence detection following the same solvent extraction procedure was used to determine if *N*-phenyl-2-naphthylamine in a formulated diet mixture degraded during storage to produce 2-naphthylamine. A feed mixture containing 5,000 ppm *N*-phenyl-2-naphthylamine was stored for 8 days at 45° C. 2-Naphthylamine was not detected in the feed mixtures at the detection level of 0.02 ppm after 8 days' storage. However, an 11% loss of *N*-phenyl-2-naphthylamine was observed.

In the 14-day and 13-week studies, the formulated diets were stored at 23° C for no longer than 2 weeks. In the 2-year studies, the formulated diets were stored protected from light at 4° C for no longer than 2 weeks.

Periodic analyses for *N*-phenyl-2-naphthylamine in feed mixtures by the same analytical methods as those used for the homogeneity studies were conducted by the study and analytical chemistry laboratories to determine if the formulated diets contained the correct concentrations of *N*-phenyl-2-naphthylamine. Formulated diets were analyzed twice during the 13-week studies; the results ranged from 92.3% to 106.2% of the target concentration (Table 2). Throughout the 2-year studies, the formulated diets were analyzed at 1- to 2-month intervals with concentrations varying from 83.4% to 107.4% of the target concentration (Table 3). The second lowest concentration observed was 90.1% of the target concentration. Because 31/32 feed mixtures analyzed were within 10% of the target concentrations, the feed mixtures were estimated to have been within specifications 97% of the time throughout the studies. Referee analyses were periodically performed by the analytical chemistry laboratory (Table 4). Good agreement was generally found between the analytical chemistry and study laboratories.

TABLE 1. PREPARATION AND STORAGE OF FORMULATED DIETS IN THE FEED STUDIES OF *N*-PHENYL-2-NAPHTHYLAMINE

Fourteen-Day Studies	Thirteen-Week Studies	Two-Year Studies
Preparation		
Weighed amount of feed mixed with weighed amount of *N*-phenyl-2-naphthylamine in a twin-shell blender and mixed for 15 min with an intensifier bar	Same as 14-d studies	Weighed amount of *N*-phenyl-2-naphthylamine layered with weighed amount of feed and mixed manually; premix mixed with additional feed in twin-shell blender for 15 min with intensifier bar for first 5 min
Maximum Storage Time		
2 wk	2 wk	2 wk
Storage Conditions		
23° C	23° C	4° C protected from light

TABLE 1. RESULTS OF ANALYSIS OF FORMULATED DIETS IN THE THIRTEEN-WEEK FEED STUDIES OF *N*-PHENYL-2-NAPHTHYLAMINE (a)

Date Mixed	Target Concentration (ppm)	Determined Concentration (ppm)	Percent of Target
06/09/80	2,500	2,540	101.6
	5,000	4,890	97.8
	10,000	10,600	106.1
	20,000	20,200	101.0
	40,000	36,900	92.3
07/28/80	2,500	2,360	94.4
	5,000	5,310	106.2
	10,000	9,850	98.5
	20,000	18,900	94.5
	40,000	40,600	101.6

(a) Results of duplicate analysis

TABLE 2. RESULTS OF ANALYSIS OF FORMULATED DIETS IN THE TWO-YEAR FEED STUDIES OF *N*-PHENYL-2-NAPHTHYLAMINE

Date Mixed	Concentration of *N*-Phenyl-2-naphthylamine in Feed for Target Concentration (a)	
	2,500 ppm	5,000 ppm
04/10/81	2,663	5,013
05/02/81	2,508 (rat)	5,005 (rat)
	2,339 (mouse)	5,192 (mouse)
06/05/81	2,553	4,765
07/17/81	(b) 2,086	4,507
09/18/81	2,281	4,808
11/20/81	2,697	5,290
01/22/82	2,594	5,371
03/18/82	2,510	4,776
05/20/82	2,450	5,065
07/13/82	2,542	4,928
09/07/82	2,412	4,731
11/03/82	2,605	5,135
12/15/82	2,670	5,267
02/16/83	2,478	5,009
04/18/83	2,525	4,953
Mean (ppm)	2,495	4,989
Standard deviation	158.1	233.3
Coefficient of variation (percent)	6.3	4.7
Range (ppm)	2,086-2,697	4,507-5,371
Number of samples	16	16

(a) Results of duplicate or triplicate analysis
(b) Out of specifications

TABLE 4. RESULTS OF REFEREE ANALYSIS OF FORMULATED DIETS IN THE TWO-YEAR FEED STUDIES OF *N*-PHENYL-2-NAPHTHYLAMINE

Date Mixed	Target Concentration (ppm)	Determined Concentration (ppm) (a)	
		Study Laboratory	Analytical Laboratory
04/10/81	5,000	(b) 5,013	5,030
11/20/81	2,500	2,597	2,467
05/20/82	5,000	5,068	5,220
11/03/82	2,500	(b) 2,606	2,590
04/19/83	5,000	(b) 4,953	5,090

(a) Results of triplicate analysis except as noted
(b) Results of duplicate analysis

FOURTEEN-DAY STUDIES

Male and female F344/N rats and B6C3F$_1$ mice were obtained from Charles River Breeding Laboratories and held for 18 days before the studies began. Animals were 7-8 weeks old when placed on study. Groups of five rats of each sex were fed diets containing 0, 3,150, 6,250, 12,500, 25,000, or 50,000 ppm *N*-phenyl-2-naphthylamine for 14 consecutive days. Groups of five mice of each sex were fed diets containing 0, 1,250, 2,500, 5,000, 10,000, or 20,000 ppm *N*-phenyl-2-naphthylamine on the same schedule. The rats and mice were observed twice daily and weighed on days 0, 8, and 15. A necropsy was performed on all animals. Details of animal maintenance are presented in Table 5.

THIRTEEN-WEEK STUDIES

Thirteen-week studies were conducted to evaluate the cumulative toxic effects of repeated administration of *N*-phenyl-2-naphthylamine and to determine the concentrations to be used in the 2-year studies. Further experimental details are summarized in Table 5.

Four-week-old male and female F344/N rats and B6C3F$_1$ mice were obtained from Charles River Breeding Laboratories, observed for 18 days, distributed to weight classes, and then assigned to cages according to a table of random numbers. Cages were assigned to dosed and control groups according to another table of random numbers.

Groups of 10 rats and 10 mice of each sex were given diets containing 0, 2,500, 5,000, 10,000, 20,000, or 40,000 ppm *N*-phenyl-2-naphthylamine for 13 weeks. Control diets consisted of NIH 07 Rat and Mouse Ration. Formulated or control diets and water were available ad libitum. Further experimental details are presented in Table 5.

Animals were checked two times per day; moribund animals were killed. Feed consumption was measured weekly by cage. Individual animal weights were taken on day 0 and recorded weekly thereafter.

At the end of the 13-week studies, survivors were killed. A necropsy was performed on all animals except those excessively autolyzed or cannibalized. Tissues and groups examined are listed in Table 5.

TWO-YEAR STUDIES

Study Design

Diets containing 0, 2,500, or 5,000 ppm *N*-phenyl-2-naphthylamine were fed to groups of 50 rats and 50 mice of each sex for 103 weeks. On the first 3 days of the study, the low dose group accidentally received 5,000 ppm in feed.

Source and Specifications of Animals

The male and female F344/N rats and B6C3F$_1$ (C57BL/6N, female × C3H/HeN MTV$^-$, male) mice used in these studies were produced under

TABLE 5. EXPERIMENTAL DESIGN AND MATERIALS AND METHODS IN THE FEED STUDIES OF *N*-PHENYL-2-NAPHTHYLAMINE

Fourteen-Day Studies	Thirteen-Week Studies	Two-Year Studies
EXPERIMENTAL DESIGN		
Size of Study Groups		
5 males and 5 females of each species	10 males and 10 females of each species	50 males and 50 females of each species
Doses		
Rats--0, 3,150, 6,250, 12,500, 25,000, or 50,000 ppm *N*-phenyl-2-naphthylamine in feed; mice--0, 1,250, 2,500, 5,000, 10,000, or 20,000 ppm *N*-phenyl-2-naphthylamine in feed	0, 2,500, 5,000, 10,000, 20,000, or 40,000 ppm *N*-phenyl-2-naphthylamine in feed	0, 2,500, or 5,000 ppm *N*-phenyl-2-naphthylamine in feed
Date of First Dose		
9/25/79	6/1/80	Rats--4/20/81; mice--5/11/81
Date of Last Dose		
10/8/79	9/1/80	Rats--4/10/83; mice--5/2/83
Duration of Dosing		
14 consecutive d	13 wk	103 wk
Type and Frequency of Observation		
Observed 2 × d; weighed by cage on d 0, 3, and 15	Observed 2 × d; individual body weights taken on d 0 and 1 × wk thereafter;	Observed 2 × d; weighed 1 × wk for 12 wk and monthly thereafter; feed consumption measured 1 × wk
Necropsy and Histologic Examination		
Necropsy performed on all animals; histologic exam not performed	Necropsy performed on all animals; histologic exams performed on all animals from control, 20,000-, and 40,000-ppm groups and on all dosed animals dying before scheduled kill; tissues examined include: adrenal glands, bone marrow, brain, colon, costochondral junction, esophagus, eyes (if grossly abnormal), gallbladder (mice), gross lesions, heart, kidneys, larynx, liver, lungs and mainstem bronchi, mandibular and mesenteric lymph nodes, pancreas, parathyroids, pituitary gland, prostate/testes/seminal vesicles or ovaries/uterus, salivary glands, sciatic nerve, skin, small intestine, spleen, stomach, thigh muscle, thymus, thyroid gland, tissue masses, trachea, and urinary bladder; liver weight/body weight ratios determined at necropsy for all groups.	Necropsy performed on all animals; complete histologic exams performed on all control and 5,000-ppm groups and on all animals dying through month 21 of studies. Tissues examined include: adrenal glands, brain, cecum, colon, duodenum, esophagus, eyes (if grossly abnormal), femur (including marrow), gallbladder (mice), gross lesions, heart, kidneys, larynx, liver, lungs and mainstem bronchi, mammary gland, mandibular and mesenteric lymph nodes, pancreas, parathyroids, pituitary gland, prostate/testes or ovaries/uterus, rectum, salivary glands, skin, small intestine, spleen, stomach, thigh muscle, thymus, thyroid gland, tissue masses, trachea, and urinary bladder; kidneys, liver, parathyroids, and thyroid gland examined for 2,500-ppm rat groups; kidneys, liver, and lung examined for 2,500-ppm male mice; kidneys examined for 2,500-ppm female mice.
ANIMALS AND ANIMAL MAINTENANCE		
Strain and Species		
F344/N rats; B6C3F$_1$ mice	F344/N rats; B6C3F$_1$ mice	F344/N rats; B6C3F$_1$ mice
Animal Source		
Charles River Breeding Laboratories (Portage, MI)	Charles River Breeding Laboratories (Kingston, NY)	Charles River Breeding Laboratories (Portage, MI)
Study Laboratory		
Battelle Columbus Laboratories	Battelle Columbus Laboratories	Battelle Columbus Laboratories

TABLE 5. EXPERIMENTAL DESIGN AND MATERIALS AND METHODS IN THE FEED STUDIES OF *N*-PHENYL-2-NAPHTHYLAMINE (Continued)

Fourteen-Day Studies	Thirteen-Week Studies	Two-Year Studies
ANIMALS AND ANIMAL MAINTENANCE (Continued)		
Method of Animal Identification		
Toe clip and ear mark	Same as 14-d studies	Rats--toe mark and ear clip; mice--toe clip and ear clip
Time Held Before Study		
18 d	18 d	17 d
Age When Placed on Study		
7-8 wk	6-7 wk	Rats--7 wk; mice--8 wk
Age When Killed		
10 wk	21 wk	Rats--111 wk; mice--112 wk
Necropsy Dates		
Rats--10/10/79; mice--10/12/79	Rats--9/2/80-9/3/80; mice--9/3/80-9/4/80	Rats--4/18/83-4/21/83; mice--5/9/83-5/12/83
Method of Animal Distribution		
Assigned from weight classes to cages according to a table of random numbers; cages assigned to study groups according to another table of random numbers	Same as 14-d studies	Same as 14-d studies
Feed		
Purina Lab Chow® (meal) (Ralston Purina Co., St. Louis, MO)	NIH 07 Rat and Mouse Ration (Zeigler Bros., Gardners, PA); available ad libitum	Same as 13-wk studies
Bedding		
Absorb-Dri® hardwood chips (Absorb-Dri, Inc., Garfield, NJ)	Absorb-Dri® hardwood chips	Absorb-Dri® hardwood chips (Absorb-Dri, Inc., Rochelle, NJ)
Water		
Automatic watering system (Edstrom Industries, Waterford, WI); available ad libitum	Same as 14-d studies	Same as 14-d studies
Cages		
Polycarbonate (Lab Products, Inc., Rochelle Park, NJ)	Same as 14-d studies	Same as 14-d studies
Cage Filters		
Spun-bonded polyester filters (Snow Filtration, Cincinnati, OH)	Same as 14-d studies	Same as 14-d studies
Animals per Cage		
5	5	5
Other Chemicals on Study in the Same Room		
None	None	None
Animal Room Environment		
Temp--70°-74° F; hum--40%-60%; fluorescent light 12 h/d; at least 15 room air changes/h	Same as 14-d studies	Temp--72°-80° F; hum--38%-80%; fluorescent light 12 h/d; at least 15 room air changes/h

strict barrier conditions at Charles River Breeding Laboratories under a contract to the Carcinogenesis Program. Breeding stock for the foundation colonies at the production facility originated at the National Institutes of Health Repository. Animals shipped for study were progeny of defined microflora-associated parents that were transferred from isolators to barrier-maintained rooms. Animals were shipped to the study laboratory at approximately 5 weeks of age. The animals were quarantined at the study laboratory for 17 days. Thereafter, a complete necropsy was performed on five animals of each sex and species to assess their health status. The rats were approximately 49 days old when placed on study, and the mice, approximately 55 days old. The health of the animals was monitored during the course of the studies according to the protocols of the NTP Sentinel Animal Program (Appendix F).

A quality control skin grafting program has been in effect since early 1978 to monitor the genetic integrity of the inbred mice used to produce the hybrid B6C3F$_1$ study animal. In mid-1981, data were obtained that showed incompatibility between the NIH C3H reference colony and the C3H colony from a Program supplier. In August 1981, inbred parental lines of mice were further tested for genetic integrity via isozyme and protein electrophoresis profiles that demonstrate phenotype expressions of known genetic loci.

The C57BL/6N mice were homogeneous at all loci tested. Eighty-five percent of the C3H mice monitored were variant at one to three loci, indicating some heterogeneity in the C3H line from this supplier. Nevertheless, the genome of this line is more homogeneous than that of randomly bred stocks.

Male mice from the C3H colony and female mice from the C57BL/6N colony were used as parents for the hyorid B6C3F$_1$ mice used in these studies. The influence of the potential genetic nonuniformity in the hybrid mice on these results is not known, but results of the studies are not affected because concurrent controls were included in each study.

Animal Maintenance

Animals were housed five per cage. Feed and water were available ad libitum. Further details of animal maintenance are given in Table 5.

Clinical Examinations and Pathology

All animals were observed two times per day. Clinical signs were recorded daily for the first 6 or 7 months for rats or mice, respectively, and monthly thereafter. Body weights by cage were recorded once per week for the first 12 weeks of the studies and approximately once per month thereafter. Mean body weights were calculated for each group. Animals found moribund and those surviving to the end of the studies were humanely killed. A necropsy was performed on all animals including those found dead, unless they were excessively autolyzed or cannibalized, missexed, or found missing. Thus, the number of animals from which particular organs or tissues were examined microscopically varies and is not necessarily equal to the number of animals that were placed on study.

During necropsy, all organs and tissues were examined for grossly visible lesions. Tissues were preserved in 10% neutral buffered formalin, embedded in paraffin, sectioned, and stained with hematoxylin and eosin. Histopathologic examination of tissues was performed according to the "inverse pyramid" design (McConnell, 1983a,b). Complete histopathologic examinations (Table 5) were performed on high dose and control animals and on low dose animals that died before the end of the studies. In addition, histopathologic examinations were performed on all grossly visible lesions in all dose groups. Potential target organs for chemically related neoplastic and nonneoplastic effects were identified from the short-term studies or the literature and were determined by examination of the pathology data; these target organs/tissues in the lower dose group were examined histopathologically. If mortality in the highest dose group exceeded that in the control group by 15%, complete histopathologic examinations were performed on all animals in the second highest dose group in addition to those in the high dose group.

When the pathology evaluation was completed, the slides, paraffin blocks, and residual wet tissues were sent to the NTP Archives for inventory, slide/block match, and wet tissue audit. The slides, individual animal data records, and pathology tables were sent to an independent quality assessment laboratory. The individual animal records and tables were compared for accuracy, slides and tissue counts were verified, and histotechnique was evaluated. All tumor diagnoses, all target tissues, and all tissues from a randomly selected 10% of the animals were evaluated by a quality assessment pathologist. The quality assessment report and slides were submitted to the Pathology Working Group (PWG) Chairperson, who reviewed all target tissues and those about which there was a disagreement between the laboratory and quality assessment pathologists.

Representative slides selected by the Chairperson were reviewed by the PWG, which includes the laboratory pathologist, without knowledge of previously rendered diagnoses. When the consensus diagnosis of the PWG differed from that of the laboratory pathologist, the laboratory pathologist was asked to reconsider the original diagnosis. This procedure has been described, in part, by Maronpot and Boorman (1982) and Boorman et al. (1985). The final diagnoses represent a consensus of contractor pathologists and the NTP Pathology Working Group. For subsequent analysis of pathology data, the diagnosed lesions for each tissue type are combined according to the guidelines of McConnell et al. (1986).

Slides/tissues are generally not evaluated in a blind fashion (i.e., without knowledge of dose group) unless the lesions in question are subtle or unless there is an inconsistent diagnosis of lesions by the laboratory pathologist. Nonneoplastic lesions are not examined routinely by the quality assessment pathologist or PWG unless they are considered part of the toxic effect of the chemical.

Statistical Methods

Data Recording: Data on this experiment were recorded in the Carcinogenesis Bioassay Data System (Linhart et al., 1974). The data elements include descriptive information on the chemicals, animals, experimental design, survival, body weight, and individual pathology results, as recommended by the International Union Against Cancer (Berenblum, 1969).

Survival Analyses: The probability of survival was estimated by the product-limit procedure of Kaplan and Meier (1958) and is presented in the form of graphs. Animals were censored from the survival analyses at the time they were found dead of other than natural causes or were found to be missing; animals dying from natural causes were not censored. Statistical analyses for a possible dose-related effect on survival used the method of Cox (1972) for testing two groups for equality and Tarone's (1975) life table test for a dose-related trend. When significant survival differences were detected, additional analyses using these procedures were carried out to determine the time point at which significant differences in the survival curves were first detected. All reported P values for the survival analysis are two-sided.

Calculation of Incidence: The incidence of neoplastic or nonneoplastic lesions is given as the ratio of the number of animals bearing such lesions at a specific anatomic site to the number of animals in which that site was examined. In most instances, the denominators include only those animals for which the site was examined histologically. However, when macroscopic examination was required to detect lesions (e.g., skin or mammary tumors) prior to histologic sampling, or when lesions could have appeared at multiple sites (e.g., lymphomas), the denominators consist of the number of animals on which a necropsy was performed.

Analysis of Tumor Incidence: Three statistical methods are used to analyze tumor incidence data. The two that adjust for intercurrent mortality employ the classical method for combining contingency tables developed by Mantel and Haenszel (1959). Tests of significance included pairwise comparisons of high dose and low dose groups with controls and tests for overall dose-response trends.

For studies in which compound administration has little effect on survival, the results of the

three alternative analyses will generally be similar. When differing results are obtained by the three methods, the final interpretation of the data will depend on the extent to which the tumor under consideration is regarded as being the cause of death. Continuity-corrected tests are used in the analysis of tumor incidence, and reported P values are one-sided.

Life Table Analysis--The first method of analysis assumed that all tumors of a given type observed in animals dying before the end of the studies were "fatal"; i.e., they either directly or indirectly caused the death of the animal. According to this approach, the proportions of tumor-bearing animals in the dosed and control groups were compared at each point in time at which an animal died with a tumor of interest. The denominators of these proportions were the total number of animals at risk in each group. These results, including the data from animals killed at the end of the studies, were then combined by the Mantel-Haenszel method to obtain an overall P value. This method of adjusting for intercurrent mortality is the life table method of Cox (1972) and of Tarone (1975). The underlying variable considered by this analysis is time to death due to tumor. If the tumor is rapidly lethal, then time to death due to tumor closely approximates time to tumor onset. In this case, the life table test also provides a comparison of the time-specific tumor incidences.

Incidental Tumor Analysis--The second method of analysis assumed that all tumors of a given type observed in animals that died before the end of the studies were "incidental"; i.e., they were merely observed at necropsy in animals dying of an unrelated cause. According to this approach, the proportions of tumor-bearing animals in dosed and control groups were compared in each of five time intervals: weeks 0-52, weeks 53-78, weeks 79-92, week 93 to the week before the terminal-kill period, and the terminal-kill period. The denominators of these proportions were the number of animals actually examined for tumors during the time interval. The individual time interval comparisons were then combined by the previously described method to obtain a single overall result. (See Haseman, 1984, for the computational details of both methods.)

Unadjusted Analyses--Primarily, survival-adjusted methods are used to evaluate tumor incidence. In addition, the results of the Fisher exact test for pairwise comparisons and the Cochran-Armitage linear trend test (Armitage, 1971; Gart et al., 1979) are given in the appendixes containing the analyses of primary tumor incidence. These two tests are based on the overall proportion of tumor-bearing animals and do not adjust for survival differences.

Historical Control Data: Although the concurrent control group is always the first and most appropriate control group used for evaluation, there are certain instances in which historical control data can be helpful in the overall assessment of tumor incidence. Consequently, control tumor incidences from the NTP historical control data base (Haseman et al., 1984, 1985) are included for those tumors appearing to show compound-related effects.

III. RESULTS

RATS

FOURTEEN-DAY STUDIES

THIRTEEN-WEEK STUDIES

TWO-YEAR STUDIES

Body Weights and Clinical Signs

Survival

Pathology and Statistical Analyses of Results

MICE

FOURTEEN-DAY STUDIES

THIRTEEN-WEEK STUDIES

TWO-YEAR STUDIES

Body Weights and Clinical Signs

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Pathology and Statistical Analyses of Results

III. RESULTS: RATS

FOURTEEN-DAY STUDIES

Three of five males and 4/5 females that received 50,000 ppm *N*-phenyl-2- naphthylamine died before the end of the studies (Table 6). The final mean body weights of rats that received 25,000 or 50,000 ppm were 36% or 57% lower than that of the controls for males and 42% or 43% lower than that of the controls for females. Arched backs, rough coats, and diarrhea were observed for males that received 12,500 ppm or more and for females that received 25,000 or 50,000 ppm.

TABLE 6. SURVIVAL AND MEAN BODY WEIGHTS OF RATS IN THE FOURTEEN-DAY FEED STUDIES OF *N*-PHENYL-2-NAPHTHYLAMINE

Concentration (ppm)	Survival (a)	Mean Body Weights (grams)			Final Weight Relative to Controls (percent)
		Initial (b)	Final	Change (c)	
MALE					
0	5/5	121	196	+75	–
3,125	5/5	120	185	+65	94.4
6,250	5/5	124	179	+55	91.3
12,500	5/5	121	161	+40	82.1
25,000	5/5	121	125	+4	63.8
50,000	(d) 2/5	118	84	−34	42.9
FEMALE					
0	5/5	108	148	+40	–
3,125	5/5	115	148	+33	101.4
6,250	5/5	111	132	+21	90.4
12,500	5/5	117	121	+4	82.9
25,000	5/5	109	85	−24	58.2
50,000	(e) 1/5	120	83	−37	56.8

(a) Number surviving/number initially in group; feed consumption data not collected.
(b) Initial mean group body weight
(c) Mean body weight change of the survivors
(d) Day of death: 10,11,12
(e) Day of death: 9,10,11,13

THIRTEEN-WEEK STUDIES

Four of 10 males and 9/10 females that received 40,000 ppm *N*-phenyl-2-naphthylamine died before the end of the studies (Table 7). The final mean body weight of rats that received 40,000 ppm was 60% lower than that of the controls for males and 44% lower for females. Feed consumption by male rats that received 40,000 ppm and female rats that received 20,000 ppm was greater than that of the controls. Rough coats were observed for rats that received 20,000 or 40,000 ppm. The liver weight to body weight ratios increased with dose and were significantly greater for males at 10,000, 20,000, or 40,000 ppm and for females at 5,000, 10,000, or 20,000 ppm than for the controls (Table 8).

Nephropathy was observed at increased incidences in dosed rats (2/10 females at 10,000 ppm, 4/10 males and 7/10 females at 20,000 ppm, and 7/10 males and 8/10 females at 40,000 ppm). Nephropathy was not seen in the remaining dose groups or in controls. The lesion consisted of degeneration of tubular epithelium and dilated tubules that contained reddish-brown granular material, remnants of tubular epithelial cells, and occasional degenerating leukocytes.

TABLE 7. SURVIVAL, MEAN BODY WEIGHTS, AND FEED CONSUMPTION OF RATS IN THE THIRTEEN-WEEK FEED STUDIES OF *N*-PHENYL-2-NAPHTHYLAMINE

Concentration (ppm)	Survival (a)	Mean Body Weights (grams) Initial (b)	Final	Change (c)	Final Weight Relative to Controls (percent)	Feed Consumption (d) Week 4	Week 13
MALE							
0	10/10	128 ± 4	323 ± 5	+195 ± 5	--	16.6	17.1
2,500	10/10	132 ± 3	327 ± 9	+195 ± 8	101	16.0	17.5
5,000	10/10	131 ± 3	294 ± 6	+163 ± 6	91	17.9	17.2
10,000	10/10	133 ± 2	277 ± 6	+144 ± 7	86	16.8	16.2
20,000	10/10	127 ± 4	245 ± 7	+118 ± 5	76	16.9	18.0
40,000	(e) 6/10	128 ± 3	128 ± 10	+4 ± 10	40	21.9	22.1
FEMALE							
0	10/10	105 ± 2	194 ± 5	+89 ± 4	--	12.7	12.1
2,500	10/10	106 ± 2	187 ± 3	+79 ± 3	96	14.0	15.0
5,000	10/10	106 ± 2	176 ± 3	+70 ± 2	91	12.6	12.3
10,000	10/10	104 ± 2	157 ± 3	+53 ± 3	81	11.2	12.2
20,000	10/10	107 ± 2	132 ± 4	+25 ± 2	68	16.4	15.7
40,000	(f) 1/10	107 ± 2	108	+16	56	(g)	(g)

(a) Number surviving/number initially in group
(b) Initial mean group body weight ± standard error of the mean; subsequent calculations based on those animals surviving to the end of the study.
(c) Mean body weight change of the survivors ± standard error of the mean
(d) Grams of feed per animal per day
(e) Week of death: 3,4,4,6
(f) Week of death: 3,3,3,3,3,3,3,3,9
(g) Not reported; too few animals remaining to provide meaningful data.

TABLE 8. ABSOLUTE AND RELATIVE LIVER WEIGHTS OF RATS IN THE THIRTEEN-WEEK FEED STUDIES OF *N*-PHENYL-2-NAPHTHYLAMINE (a)

Concentration (ppm)	No. Livers Examined	Body Weight (grams) (b)	Liver Weight (mg)	Liver Weight/Body Weight Ratio (mg/g)
MALE				
0	10	327 ± 9.5	14,470 ± 380	44.7 ± 1.87
2,500	10	331 ± 7.8	15,642 ± 662	47.1 ± 1.14
5,000	10	313 ± 6.6	15,657 ± 447	50.2 ± 1.53
10,000	9	(c) 285 ± 7.9	(c) 17,238 ± 444	(c) 60.8 ± 2.11
20,000	10	(c) 257 ± 11.4	(d) 16,907 ± 647	(c) 65.9 ± 2.00
40,000	5	(c) 140 ± 11.4	(d) 11,836 ± 658	(c) 85.4 ± 3.33
FEMALE				
0	10	198 ± 5.0	7,790 ± 320	39.2 ± 1.23
2,500	10	186 ± 2.8	7,943 ± 273	42.5 ± 0.96
5,000	10	(d) 181 ± 3.3	8,007 ± 264	(d) 44.1 ± 0.88
10,000	10	(c) 161 ± 3.4	(c) 9,239 ± 286	(c) 57.6 ± 1.40
20,000	10	(c) 141 ± 4.2	(c) 9,775 ± 276	(c) 69.3 ± 1.39
(e) 40,000	1	117	9,571	81.4

(a) Mean ± standard error; P values versus the controls by Dunnett's test (Dunnett, 1955).
(b) Body weights were taken at necropsy, 1-2 days after last day of dosing.
(c) $P<0.01$
(d) $P<0.05$
(e) Not included in statistical analysis

Hematopoietic hypoplasia or atrophy of the femoral bone marrow was seen in 7/10 males and 8/10 females at 40,000 ppm and in 2/10 females at 20,000 ppm. Testicular hypospermatogenesis was observed in 2/10 males that received 40,000 ppm. Lymphoid degeneration of the thymus was observed in 4/10 males and 7/10 females that received 40,000 ppm. Lymphoid depletion of the spleen was observed in 2/10 males and 6/10 females that received 40,000 ppm. The lesions in the bone marrow, testis, thymus, and spleen occurred primarily in animals that died or that had marked reduction in body weight.

Dose Selection Rationale: Because of increased mortality, lower weight gain, and kidney lesions seen at higher concentrations in the 13-week studies, dietary concentrations of *N*-phenyl-2-naphthylamine selected for rats for the 2-year studies were 2,500 and 5,000 ppm.

TWO-YEAR STUDIES

Body Weights and Clinical Signs

Final mean body weights relative to controls were 12% and 16% lower than that of controls for low and high dose male rats and 15% and 31% lower for low and high dose female rats (Table 9 and Figure 3). The estimated average daily feed consumption per rat was 94% and 97% that of the controls for low and high dose males and 88% that of the controls for low and high dose females (Appendix G, Tables G1 and G2). The average amount of *N*-phenyl-2-naphthylamine consumed per day was approximately 103 mg/kg and 225 mg/kg for low and high dose male rats, respectively, and 118 mg/kg and 261 mg/kg for low and high dose female rats. Ninety percent to 100% of the dosed female rats had brown stains in the urogenital region.

TABLE 9. MEAN BODY WEIGHTS AND SURVIVAL OF RATS IN THE TWO-YEAR FEED STUDIES OF *N*-PHENYL-2-NAPHTHYLAMINE

Weeks on Study	Control		2,500 ppm			5,000 ppm		
	Av. Wt. (grams)	No. of Survivors	Av. Wt. (grams)	Wt. (percent of controls)	No. of Survivors	Av. Wt. (grams)	Wt. (percent of controls)	No. of Survivors
MALE								
0	127	50	127	100	50	126	99	50
1	155	50	175	99	50	156	99	50
2	183	50	195	99	50	191	98	50
3	208	50	212	98	50	211	98	50
4	232	50	228	98	50	231	99	50
5	247	50	238	96	50	244	98	50
6	267	50	258	96	50	265	99	50
7	285	50	279	98	50	283	99	50
8	299	50	297	99	50	295	98	50
9	313	50	307	98	50	308	98	50
10	326	50	318	98	50	321	98	50
11	335	50	329	98	50	331	99	50
12	348	50	339	97	50	341	98	50
13	358	50	348	97	50	350	98	50
16	371	50	360	97	50	366	99	50
21	388	50	377	97	50	386	99	50
25	414	50	394	95	50	397	96	50
30	436	50	415	95	50	418	96	50
34	451	50	430	95	50	433	96	50
37	467	50	451	97	50	451	97	50
43	486	50	464	96	50	467	96	50
48	498	50	480	96	50	483	97	50
52	513	50	490	96	50	494	96	50
56	525	50	500	95	50	504	96	50
60	537	50	510	95	50	513	96	50
64	547	50	521	95	50	524	96	50
68	557	50	531	95	50	534	96	50
72	567	50	541	95	50	544	96	50
77	576	50	550	95	50	553	96	50
81	585	50	559	96	50	562	96	50
85	593	50	567	96	50	570	96	50
91	599	50	573	96	50	576	96	50
95	603	50	577	96	50	580	96	50
99	606	50	580	96	50	583	96	50
103-104	608	38	582	96	50	585	96	37
FEMALE								
0	121	50	120	99	50	119	98	50
1	140	50	135	96	50	134	96	50
2	158	50	141	89	50	148	94	50
3	168	50	151	90	50	158	94	50
4	177	50	159	90	50	168	95	50
5	186	50	167	90	50	177	95	50
6	193	50	173	90	50	181	94	50
7	199	50	179	90	50	188	94	50
8	204	50	181	89	50	192	94	50
9	209	50	185	89	50	196	94	50
10	214	50	188	88	50	199	93	50
11	218	50	191	88	50	202	93	50
12	224	50	197	88	50	208	93	50
13	231	50	202	88	50	213	92	50
16	241	50	212	88	50	223	93	50
21	256	50	225	88	50	236	92	50
25	271	50	238	88	50	248	92	50
30	287	50	252	88	50	262	91	50
34	297	50	261	88	50	271	91	50
37	305	50	268	88	50	278	91	50
43	317	50	279	88	50	289	91	50
48	326	50	287	88	50	297	91	50
52	333	50	293	88	50	303	91	50
56	339	50	298	88	50	308	91	50
60	345	50	303	88	50	313	91	50
64	350	50	308	88	50	318	91	50
68	355	50	312	88	50	322	91	50
72	359	50	316	88	50	326	91	50
77	363	50	319	88	50	329	91	50
81	366	50	322	88	50	332	91	50
85	369	50	325	88	50	335	91	50
91	371	50	327	88	50	337	91	50
95	373	50	329	88	50	339	91	50
99	375	50	331	88	50	341	91	50
103-104	376	37	332	88	50	342	91	47

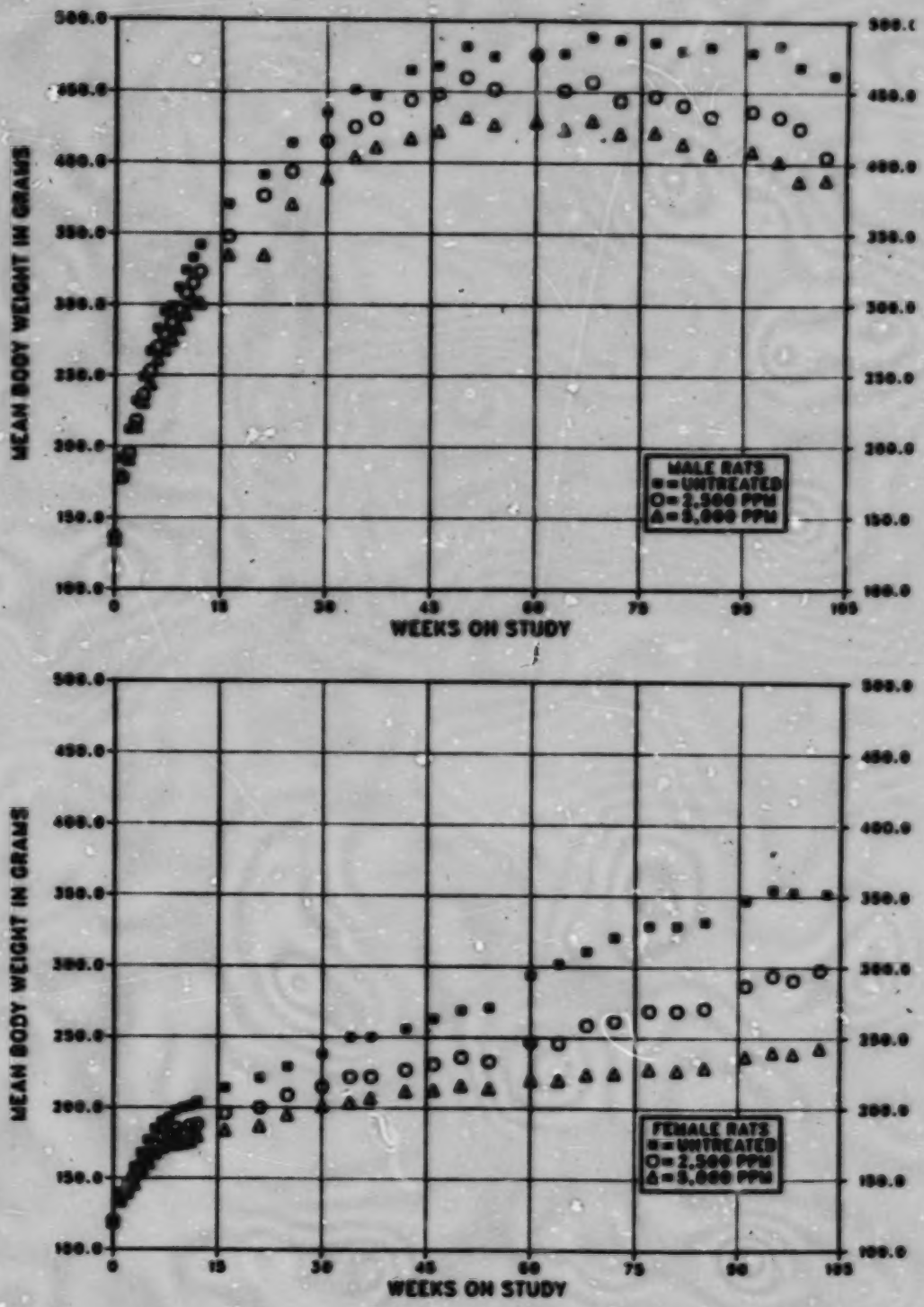

FIGURE 3. GROWTH CURVES FOR RATS FED DIETS CONTAINING *N*-PHENYL-2-NAPHTHYLAMINE FOR TWO YEARS

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Survival

Estimates of the probabilities of survival for male and female rats fed diets containing *N*-phenyl-2-naphthylamine at the concentrations used in these studies and for controls are shown in Table 10 and in the Kaplan and Meier curves in Figure 4. The survival of the high dose group of male rats was greater ($P<0.05$) than that of the controls after week 101.

Pathology and Statistical Analyses of Results

This section describes the significant or noteworthy changes in the incidences of rats with neoplastic or nonneoplastic lesions of the kidney, spleen, cecum, colon, thyroid gland, mammary gland, hematopoietic system, pituitary gland, and parathyroids.

Lesions in male rats are summarized in Appendix A. Histopathologic findings on neoplasms are summarized in Table A1. Table A2 gives the survival and tumor status for individual male rats. Table A3 contains the statistical analyses of those primary tumors that occurred with an incidence of at least 5% in one of the three groups. The statistical analyses used are discussed in Chapter II (Statistical Methods) and Table A3 (footnotes). Historical incidences of tumors in control male rats are listed in Table A4. Findings on nonneoplastic lesions are summarized in Table A5.

Lesions in female rats are summarized in Appendix B. Histopathologic findings on neoplasms are summarized in Table B1. Table B2 gives the survival and tumor status for individual female rats. Table B3 contains the statistical analyses of those primary tumors that occurred with an incidence of at least 5% in one of the three groups. The statistical analyses used are discussed in Chapter II (Statistical Methods) and Table B3 (footnotes). Historical incidences of tumors in control female rats are listed in Table B4. Findings on nonneoplastic lesions are summarized in Table B5.

TABLE 10. SURVIVAL OF RATS IN THE TWO-YEAR FEED STUDIES OF *N*-PHENYL-2-NAPHTHYLAMINE

	Control	2,500 ppm	5,000 ppm
MALE (a)			
Animals initially in study	50	50	50
Nonaccidental deaths before termination (b)	26	22	16
Killed at termination	24	28	34
Survival P values (c)	0.035	0.334	0.047
FEMALE (a)			
Animals initially in study	50	50	50
Nonaccidental deaths before termination (b)	14	6	12
Killed at termination	36	44	38
Survival P values (c)	0.683	0.082	0.775

(a) Terminal-kill period: week 104
(b) Includes animals killed in a moribund condition
(c) The result of the life table trend test is in the control column, and the results of the life table pairwise comparisons with the controls are in the dosed columns.

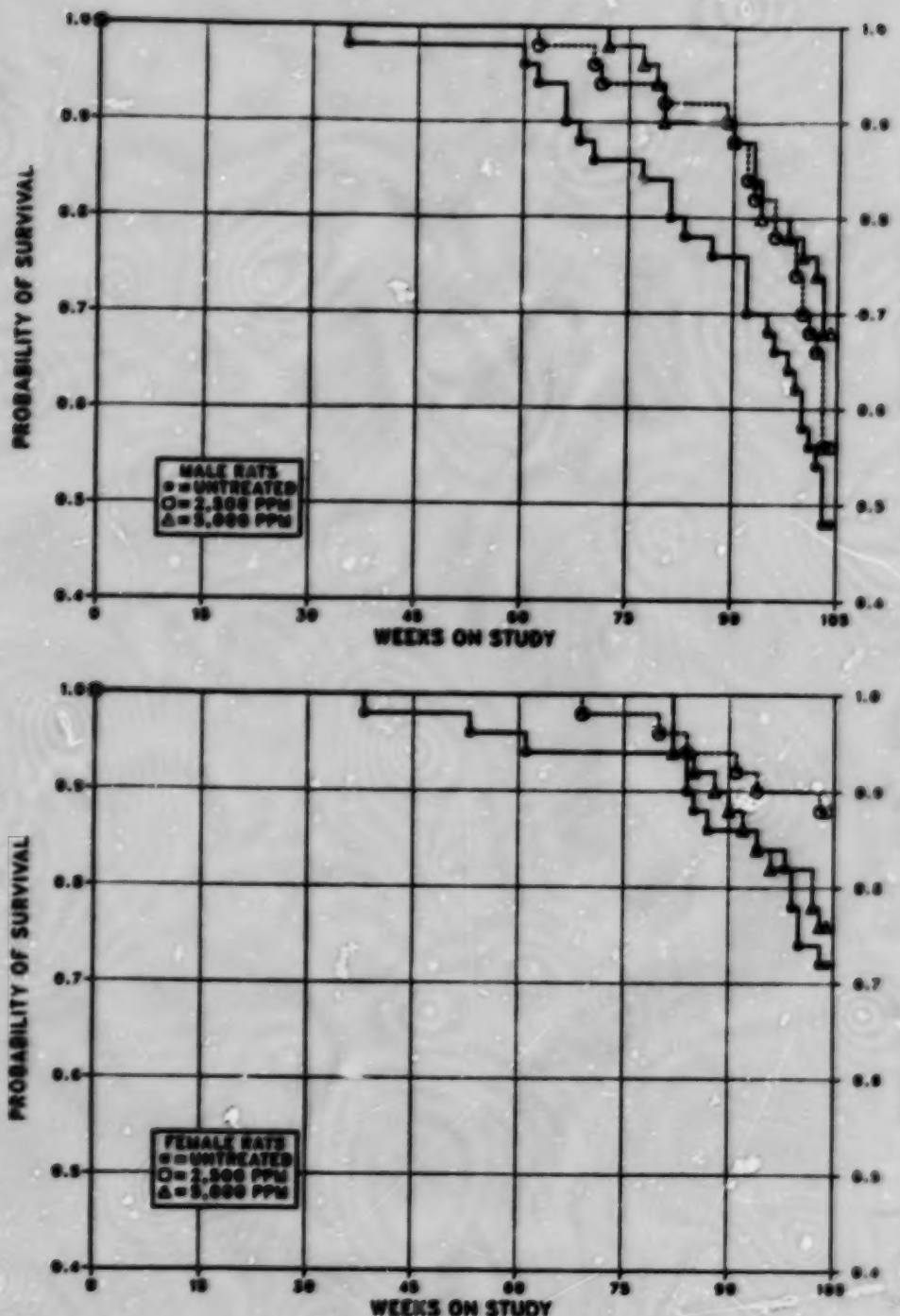

FIGURE 4. KAPLAN-MEIER SURVIVAL CURVES FOR RATS FED DIETS CONTAINING *N*-PHENYL-2-NAPHTHYLAMINE FOR TWO YEARS

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Kidney: Chemically related nonneoplastic lesions were seen in the kidney of male and female rats (Table 11). The lesions were more extensive and severe in female rats than in males and consisted of hydronephrosis (dilatation of the renal pelvis), hyperplasia of the epithelium lining the pelvis, necrosis of the renal papilla, atrophy of tubules, interstitial fibrosis, and acute and chronic inflammation. Calculi also were observed in the renal pelvis of many low and high dose females; they consisted of a yellow material unlike the urolith occasionally occurring spontaneously in aged rats. The calculi may represent an excreted metabolite of the chemical which precipitated in the concentrated urine of the pelvis. In male rats, the chemically related lesion consisted primarily of acute suppurative inflammation, but the degree of severity of nephropathy also was judged to be slightly higher in the dosed male rats than in the controls. Neoplasms (adenomas or adenocarcinomas) were seen in three control males, one low dose male, and one high dose male; none was observed in females.

TABLE 11. NUMBER OF RATS WITH SELECTED RENAL LESIONS IN THE TWO-YEAR FEED STUDIES OF *N*-PHENYL-2-NAPHTHYLAMINE

Site/Lesion	Male			Female		
	Control	2,500 ppm	5,000 ppm	Control	1,250 ppm	2,500 ppm
Number examined	50	50	50	50	50	50
Kidney						
Mineralization	1	2	2	0	20	23
Hydronephrosis	2	0	0	0	1	47
Cyst, NOS	0	5	5	0	0	4
Chronic focal inflammation	0	0	0	0	4	41
Atrophy	0	0	0	0	1	32
Kidney/Interstitium						
Multifocal fibrosis	0	0	0	0	0	43
Renal Papilla						
Necrosis, NOS	0	0	0	0	7	9
Kidney/Tubule						
Acute suppurative inflammation	8	32	40	3	4	33
Kidney/Pelvis						
Calculus	0	0	0	0	12	11
Epithelial hyperplasia	1	2	1	2	13	40

Spleen: A fibrosarcoma was observed in one low dose male rat, and a sarcoma was observed in one high dose male rat. No fibrosarcomas and five sarcomas have been previously diagnosed in the spleen of 1,954 (0.3%) untreated control male F344/N rats in NTP studies.

Cecum and Colon: A fibrosarcoma was observed in the cecum of one low dose male rat and in the colon of a second low dose male rat. Fibrosarcomas were not previously diagnosed in the large intestine of 1,879 untreated control male F344/N rats in NTP studies. One fibroma was diagnosed in 1,879 (0.05%) untreated control male F344/N rats in NTP studies.

Thyroid Gland: C-Cell adenomas and C-cell carcinomas in female rats and C-cell adenomas or carcinomas (combined) in male and female rats occurred with significant negative trends; the incidences of C-cell adenomas in dosed female rats and of C-cell adenomas or carcinomas (combined) in high dose male rats and dosed female rats were significantly lower than those in the controls (Table 12). The incidence in the high dose male rats was significant by the life table test only.

Mammary Gland: Fibroadenomas in female rats occurred with a significant negative trend; the incidences in the dosed groups were significantly lower than that in the controls (Table 12).

Hematopoietic System: The incidence of mononuclear cell leukemia in high dose female rats was significantly lower than that in controls by the life table test (Table 12).

Pituitary Gland: The incidence of adenomas in high dose female rats was significantly lower than that in controls (Table 12).

Parathyroids: Hyperplasia was observed at an increased incidence in high dose male rats (male: control, 2/39, 5%; low dose, 4/43, 9%; high dose, 9/43, 21%; female: 1/38, 3%; 2/44, 5%; 4/41, 10%). The increased incidences are likely due to the increased severity of nephropathy in dosed male and female rats.

TABLE 12. REDUCTION IN THE INCIDENCE OF THYROID GLAND, MAMMARY GLAND, HEMATOPOIETIC SYSTEM, AND PITUITARY GLAND LESIONS IN DOSED RATS IN THE TWO-YEAR FEED STUDIES OF *N*-PHENYL-2-NAPHTHYLAMINE (a)

	Control	2,500 ppm (b)	5,000 ppm (b)
MALE			
Thyroid Gland			
Hyperplasia	38/49 (78%)	39/50 (78%)	43/49 (88%)
Adenoma	7/49 (14%)	7/50 (14%)	4/49 (8%)
Carcinoma	2/49 (4%)	0/50 (0%)	0/49 (0%)
Adenoma or Carcinoma (c)	(d) 9/49 (18%)	7/50 (14%)	(e) 4/49 (8%)
FEMALE			
Thyroid Gland			
C-Cell Hyperplasia	43/50 (86%)	41/49 (84%)	43/50 (86%)
C-Cell Adenoma	(d) 17/50 (34%)	(e) 11/49 (22%)	(f) 1/50 (2%)
C-Cell Carcinoma	(d) 3/50 (6%)	0/49 (0%)	0/50 (0%)
C-Cell Adenoma or Carcinoma (g)	(d) 19/50 (38%)	(f) 11/49 (22%)	(f) 1/50 (2%)
Mammary Gland			
Cystic Hyperplasia	42/50 (84%)	(h) 2/7 (29%)	41/50 (82%)
Fibroadenoma (i,j)	(d) 16/50 (32%)	(f) 5/50 (10%)	(f) 5/50 (10%)
Hematopoietic System			
Leukemia (k)	14/50 (28%)	6/50 (12%)	(e) 6/50 (12%)
Pituitary Gland (pars distalis)			
Hyperplasia	11/50 (22%)	5/25 (20%)	14/49 (29%)
Adenoma	31/50 (62%)	16/25 (64%)	(f) 14/49 (29%)
Carcinoma	1/50 (2%)	0/25 (0%)	0/49 (0%)
Adenoma or Carcinoma (l)	32/50 (64%)	16/25 (64%)	(f) 14/49 (29%)

(a) The statistical analyses used are discussed in Chapter II (Statistical Methods) and Appendix A, Table A3 (footnotes).
(b) The estimated dose in milligrams per kilogram per day is given in Chapter III (Body Weights and Clinical Signs) and in Appendix G.
(c) Historical incidence at study laboratory (mean ± SD): 25/336 (7% ± 4%); historical incidence in NTP studies: 192/1,928 (10% ± 6%)
(d) Negative trend ($P<0.05$)
(e) Lower than control ($P<0.05$, life table test)
(f) Lower than control ($P<0.05$)
(g) Historical incidence at study laboratory (mean ± SD): 16/330 (5% ± 3%); historical incidence in NTP studies: 182/1,952 (9% ± 5%)
(h) Denominator is number of animals with mammary gland examined microscopically.
(i) Denominator is number of animals for which a necropsy was performed.
(j) Historical incidence at study laboratory (mean ± SD): 58/337 (17% ± 5%); historical incidence in NTP studies: 562/2,021 (28% ± 11%)
(k) Historical incidence at study laboratory (mean ± SD): 58/337 (17% ± 3%); historical incidence in NTP studies: 375/2,021 (19% ± 7%)
(l) Historical incidence at study laboratory (mean ± SD): 151/312 (48% ± 11%); historical incidence in NTP studies: 931/1,952 (48% ± 11%)

III. RESULTS: MICE

FOURTEEN-DAY STUDIES

All the mice lived to the end of the studies (Table 13). The final mean body weights of mice that received 10,000 or 20,000 ppm *N*-phenyl-2-naphthylamine were 6% or 12% lower than that of the controls for males and 8% lower for females. No compound-related clinical signs of toxicity were observed. Because no toxic effects were observed in these 14-day studies, doses of 0, 2,500, 5,000, 10,000, 20,000, and 40,000 ppm were selected for the 13-week studies.

TABLE 13. SURVIVAL AND MEAN BODY WEIGHTS OF MICE IN THE FOURTEEN-DAY FEED STUDIES OF *N*-PHENYL-2-NAPHTHYLAMINE

Concentration (ppm)	Survival (a)	Mean Body Weights (grams) Initial	Final	Change (b)	Final Weight Relative to Controls (percent)
MALE					
0	5/5	27.0	28.8	+1.8	--
1,250	5/5	25.8	27.0	+1.2	93.8
2,500	5/5	25.8	28.4	+2.6	98.6
5,000	5/5	26.6	28.0	+1.4	97.2
10,000	5/5	26.0	27.0	+1.0	93.8
20,000	5/5	25.8	25.4	−0.2	88.2
FEMALE					
0	5/5	19.6	22.6	+3.0	--
1,250	5/5	20.6	23.2	+2.6	102.7
2,500	5/5	20.0	20.8	+0.8	92.0
5,000	5/5	20.0	22.0	+2.0	97.3
10,000	5/5	19.8	20.8	+1.0	92.0
20,000	5/5	20.2	20.8	+0.6	92.0

(a) Number surviving/number initially in group; feed consumption data not collected.
(b) Mean body weight change of the group

THIRTEEN-WEEK STUDIES

Two of 10 male mice and 7/10 female mice that received 40,000 ppm died before the end of the studies (Table 14). Deaths of mice in other dosed groups were not considered to be compound related. The final mean body weights of mice that received 10,000, 20,000, or 40,000 ppm were 15%, 14%, or 32% lower, respectively, than that of the controls for males and 12%, 9%, or 25% lower for females. Feed consumption data indicated that feed was scattered. No compound-related clinical signs were observed in animals that lived to the end of the studies. The liver weight to body weight ratios increased with increasing dose and were significantly greater than those of the controls for male mice that received 10,000, 20,000, or 40,000 ppm and for female mice at 20,000 or 40,000 ppm (Table 15). The low values for body weights, liver weights, and liver weight to body weight ratios for female mice fed 10,000 ppm cannot be explained from the available data.

Nephropathy was observed at increased incidences and severity in dosed mice (male: control, 0/10; 5,000 ppm, 0/10; 10,000 ppm, 7/10; 20,000 ppm, 10/10; 40,000 ppm, 10/10; female: control, 0/9; 5,000 ppm, 2/10; 10,000 ppm, 3/10; 20,000 ppm, 9/9; 40,000 ppm, 8/8). The lesions were characterized by dilated tubules in the cortex, necrotic epithelium, and regeneration of the tubular epithelial cells.

TABLE 14. SURVIVAL, MEAN BODY WEIGHTS, AND FEED CONSUMPTION OF MICE IN THE THIRTEEN-WEEK FEED STUDIES OF *N*-PHENYL-2-NAPHTHYLAMINE

Concentration (ppm)	Survival (a)	Mean Body Weights (grams) Initial (b)	Final	Change (c)	Final Weight Relative to Controls (percent)	Feed Consumption (d) Week 4	Week 13
MALE							
0	10/10	23.9 ± 0.6	33.5 ± 0.8	+9.6 ± 0.5	–	8.2	6.5
2,500	9/10	23.8 ± 0.4	32.8 ± 1.0	+9.0 ± 0.9	97.9	8.2	9.6
5,000	9/10	24.7 ± 0.4	33.0 ± 0.6	+8.1 ± 0.5	98.5	7.9	7.2
10,000	9/10	24.9 ± 0.4	28.3 ± 0.5	+3.3 ± 0.5	84.5	7.8	9.4
20,000	9/10	25.1 ± 0.4	28.7 ± 1.0	+3.7 ± 1.0	85.7	7.3	6.8
40,000	(e) 8/10	24.7 ± 0.2	22.9 ± 0.4	−1.9 ± 0.4	68.4	7.9	8.7
FEMALE							
0	10/10	18.4 ± 0.3	27.1 ± 0.8	+8.7 ± 0.7	–	8.3	6.8
2,500	10/10	18.5 ± 0.5	25.9 ± 0.8	+7.4 ± 0.6	95.6	8.8	7.4
5,000	10/10	17.8 ± 0.3	25.0 ± 0.5	+7.2 ± 0.3	92.3	9.1	6.7
10,000	10/10	18.3 ± 0.3	23.8 ± 0.4	+5.5 ± 0.4	87.8	8.4	6.0
20,000	10/10	18.3 ± 0.4	24.7 ± 1.0	+6.4 ± 1.0	91.1	7.5	5.7
40,000	(f) 3/10	17.9 ± 0.4	20.3 ± 0.3	+2.7 ± 0.9	74.9	7.6	8.8

(a) Number surviving/number initially in group
(b) Initial mean group body weight ± standard error of the mean; subsequent calculations based on those animals surviving to the end of the study.
(c) Mean body weight change of the survivors ± standard error of the mean
(d) Grams of feed per animal per day
(e) Week of death: 6,12
(f) Week of death: 2,5,7,10,10,10,10

TABLE 15. ABSOLUTE AND RELATIVE LIVER WEIGHTS FOR MICE IN THE THIRTEEN-WEEK FEED STUDIES OF *N*-PHENYL-2-NAPHTHYLAMINE (a)

Concentration (ppm)	No. Livers Examined	Necropsy Body Weight (grams)	Liver Weight (mg)	Liver Weight/ Necropsy Body Weight Ratio (mg/g)
MALE				
0	10	33.1 ± 1.16	1,784 ± 83	53.8 ± 1.67
2,500	9	(b) 28.6 ± 1.42	(b) 1,363 ± 142	46.9 ± 3.06
5,000	9	34.2 ± 0.46	2,096 ± 82	61.2 ± 2.03
10,000	(c) 5	30.0 ± 0.71	2,051 ± 113	(b) 67.0 ± 4.25
20,000	8	30.9 ± 1.12	(b) 2,233 ± 109	(d) 72.4 ± 2.53
40,000	8	(d) 26.5 ± 0.84	(d) 2,225 ± 74	(d) 83.3 ± 3.49
FEMALE				
0	10	28.5 ± 0.72	1,548 ± 65	54.3 ± 1.66
2,500	10	(d) 26.0 ± 0.58	1,384 ± 74	53.0 ± 2.04
5,000	10	26.9 ± 0.64	1,481 ± 60	55.0 ± 1.48
10,000	9	(d) 17.9 ± 0.35	(d) 742 ± 63	(d) 41.2 ± 2.77
20,000	10	(d) 23.7 ± 0.21	1,564 ± 48	(d) 65.5 ± 1.80
40,000	3	(d) 22.3 ± 0.33	1,775 ± 79	(d) 79.6 ± 4.66

(a) Mean ± standard error; P values versus the controls by Dunnett's test (Dunnett, 1955).
(b) $P<0.05$
(c) One body weight not taken at necropsy; ratio is based on five animals; six livers were examined.
(d) $P<0.01$

Dose Selection Rationale: Because of lower weight gain and kidney lesions seen at higher concentrations in the 13-week studies, dietary concentrations of *N*-phenyl-2-naphthylamine selected for mice for the 2-year studies were 2,500 ppm and 5,000 ppm.

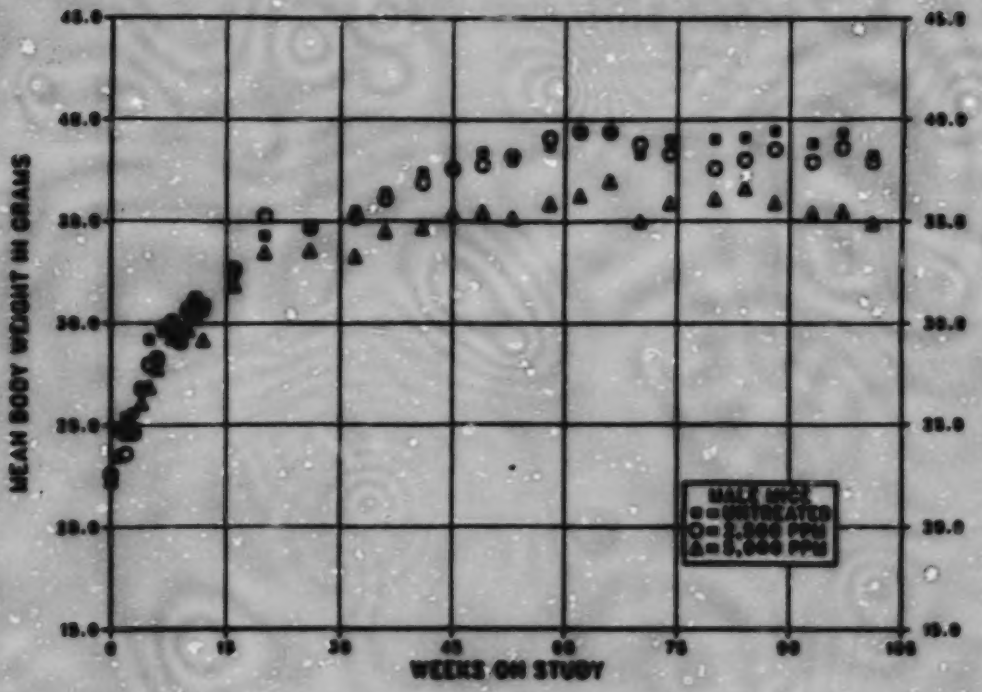
TWO-YEAR STUDIES

Body Weights and Clinical Signs

Mean body weights of high dose male mice were 5%-10% lower than those of the controls after week 36 (Table 16 and Figure 5). Mean body weights of low dose and control male mice were comparable. Mean body weights of high dose female mice were 7%-13% lower than those of the controls between weeks 20 and 45 and 14%-26% lower thereafter. Mean body weights of low dose female mice were within 7% of those of the controls throughout the study. The average daily feed consumption by low and high dose male mice was 110% that of the controls and by low and high dose female mice, 106% and 98%, respectively, that of the controls (Appendix G, Tables G3 and G4). The average amount of *N*-phenyl-2-naphthylamine consumed per day was approximately 500 mg/kg and 1,000 mg/kg for low and high dose males, respectively, and 450 mg/kg and 900 mg/kg for low and high dose females. No compound-related clinical signs were observed.

TABLE 18. MEAN BODY WEIGHTS AND SURVIVAL OF MICE IN THE TWO-YEAR FEED STUDIES OF *N*-PHENYL-2-NAPHTHYLAMINE

Weeks on Study	Control		2,500 ppm			5,000 ppm		
	Av. Wt. (grams)	No. of Survivors	Av. Wt. (grams)	Wt. (percent of controls)	No. of Survivors	Av. Wt. (grams)	Wt. (percent of controls)	No. of Survivors
MALE								
0	22.4	50	22.6	101	50	22.3	100	50
1	24.7	50	24.8	100	50	23.5	95	50
2	25.4	50	25.6	101	50	24.6	97	50
3	25.6	50	25.6	100	50	24.9	97	50
4	26.8	50	26.8	100	50	26.1	97	50
5	28.2	50	28.0	99	50	25.9	92	50
6	28.1	50	28.3	101	50	27.8	99	50
7	28.6	49	28.8	101	49	28.7	100	50
8	28.6	49	29.1	102	49	28.3	99	50
9	29.5	49	29.1	99	49	28.3	96	50
10	30.8	49	30.6	99	49	29.7	96	50
11	31.3	49	31.1	99	49	30.3	97	50
12	30.0	49	30.9	101	49	29.2	96	50
16	32.5	48	32.7	101	48	31.9	98	49
20	34.3	48	33.3	100	47	33.5	98	49
24	34.7	48	34.7	100	45	33.6	97	48
28	35.5	48	35.2	99	45	33.3	94	47
32	36.6	48	36.1	99	45	34.5	95	44
36	37.4	48	36.9	99	45	34.7	93	43
41	37.5	47	37.6	100	45	35.4	94	43
45	38.4	47	37.8	98	45	35.5	93	43
49	38.0	47	38.3	101	45	35.3	93	43
53	38.7	44	38.1	101	45	35.9	93	41
58	38.5	43	38.4	100	44	36.3	95	41
62	38.4	43	38.4	100	44	37.0	96	39
70	38.3	41	38.8	101	43	36.0	94	39
74	38.0	41	38.3	99	43	35.9	94	39
80	38.9	40	37.8	98	41	36.1	93	39
84	39.1	40	38.0	97	41	35.6	91	37
88	38.4	37	38.5	98	39	35.9	94	34
93	38.0	36	37.9	98	39	35.4	93	34
97	38.3	35	38.6	98	38	35.5	93	31
101	38.2	33	37.9	99	38	34.9	91	30
FEMALE								
0	17.8	50	17.1	96	50	18.5	104	50
1	19.7	50	19.5	99	50	19.6	99	50
2	20.4	50	20.1	98	50	19.5	96	50
3	21.3	50	20.6	97	50	20.3	95	50
4	22.3	50	21.6	97	50	21.3	96	50
5	22.4	50	21.6	97	50	21.6	96	50
6	22.6	50	22.3	99	50	21.6	96	50
7	23.5	50	22.9	97	50	22.8	97	50
8	23.4	50	23.1	99	50	23.0	98	50
9	23.5	50	23.3	99	50	21.6	92	50
10	24.1	50	23.5	98	50	23.4	97	50
11	24.4	50	23.7	97	50	23.6	97	50
12	24.5	50	23.6	96	50	23.5	96	50
16	25.7	50	25.5	99	50	24.9	97	50
20	27.6	50	26.7	97	50	25.6	93	50
24	28.3	50	27.8	98	50	26.0	92	50
28	28.7	50	27.6	97	50	26.3	92	49
32	28.7	50	28.7	97	49	27.5	96	47
36	28.7	50	28.1	98	49	27.1	94	47
41	31.0	50	31.5	101	49	28.4	92	47
45	30.1	50	31.5	105	49	28.5	95	47
49	30.2	50	31.0	103	49	27.7	92	47
53	30.1	50	30.1	100	49	28.4	94	47
58	30.3	50	30.1	100	49	28.0	93	47
62	30.3	50	30.1	99	49	28.4	94	47
70	30.6	50	30.3	99	48	28.4	93	47
74	31.0	50	30.0	97	48	28.5	92	47
80	31.1	49	30.0	96	47	31.3	101	46
84	30.7	48	29.1	95	45	28.3	92	46
88	30.1	46	29.5	98	45	28.0	93	45
93	30.4	43	29.3	96	43	28.1	92	43
97	31.1	41	29.1	94	42	28.6	92	42
101	30.6	39	30.0	98	38	30.6	100	39



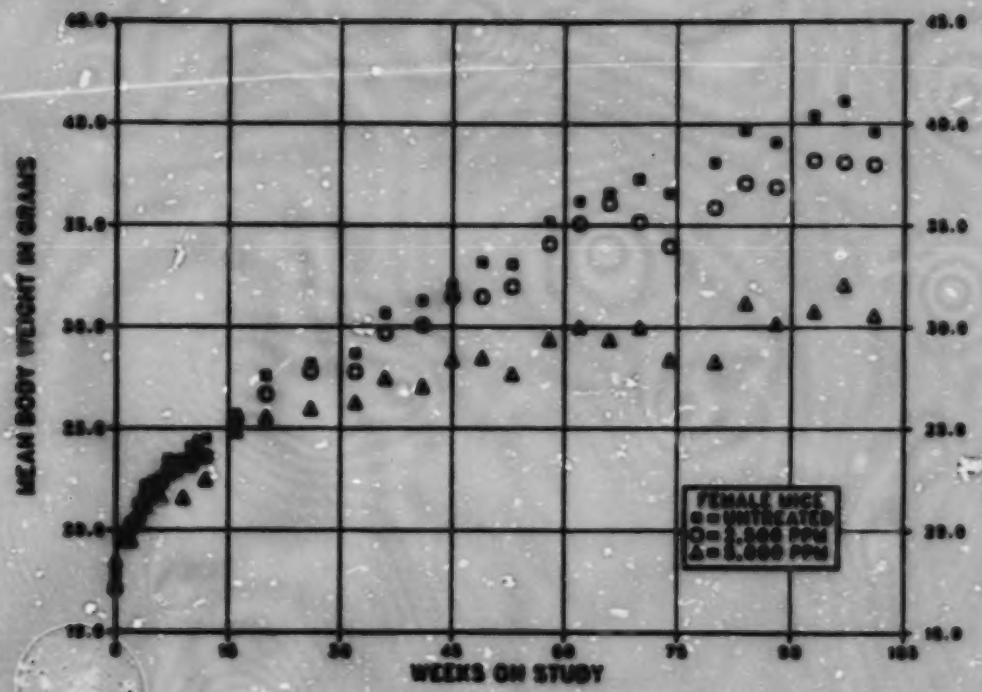


FIGURE 5. GROWTH CURVES FOR MICE FED DIETS CONTAINING *N*-PHENYL-2-NAPHTHYLAMINE FOR TWO YEARS

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Survival

Estimates of the probabilities of survival for male and female mice fed diets containing *N*-phenyl-2-naphthylamine at the concentrations used in these studies and for controls are shown in Table 17 and in the Kaplan and Meier curves in Figure 6. No significant differences in survival were observed between any groups of either sex. Two high dose male and two high dose female mice were found dead during week 33. These deaths were recorded as natural deaths; laboratory notes suggest that these may have resulted from dehydration.

Pathology and Statistical Analyses of Results

This section describes the significant or noteworthy changes in the incidences of mice with neoplastic or nonneoplastic lesions of the kidney, liver, subcutaneous tissue, ovary, and uterus.

Lesions in male mice are summarized in Appendix C. Histopathologic findings on neoplasms are summarized in Table C1. Table C2 gives the survival and tumor status for individual male mice. Table C3 contains the statistical analyses of those primary tumors that occurred with an incidence of at least 5% in one of the three groups. The statistical analyses used are discussed in Chapter II (Statistical Methods) and Table C3 (footnotes). Historical incidences of tumors in control male mice are listed in Table C4. Findings on nonneoplastic lesions are summarized in Table C5.

Lesions in female mice are summarized in Appendix D. Histopathologic findings on neoplasms are summarized in Table D1. Table D2 gives the survival and tumor status for individual female mice. Table D3 contains the statistical analyses of those primary tumors that occurred with an incidence of at least 5% in one of the three groups. The statistical analyses used are discussed in Chapter II (Statistical Methods) and Table D3 (footnotes). Historical incidences of tumors in control female mice are listed in Table D4. Findings on nonneoplastic lesions are summarized in Table D5.

TABLE 17. SURVIVAL OF MICE IN THE TWO-YEAR FEED STUDIES OF *N*-PHENYL-2-NAPHTHYLAMINE

	Control	2,500 ppm	5,000 ppm
MALE (a)			
Animals initially in study	50	50	50
Nonaccidental deaths before termination (b)	16	14	22
Animals missing	1	0	0
Killed at termination	33	36	28
Survival P values (c)	0.251	0.784	0.291
FEMALE (a)			
Animals initially in study	50	50	50
Nonaccidental deaths before termination (b)	14	20	15
Killed at termination	36	29	34
Died during termination period	0	1	1
Survival P values (c)	0.845	0.285	0.925

(a) Terminal-kill period: week 104
(b) Includes animals killed in a moribund condition
(c) The result of the life table trend test is in the control column, and the results of the life table pairwise comparisons with the controls are in the dosed columns.

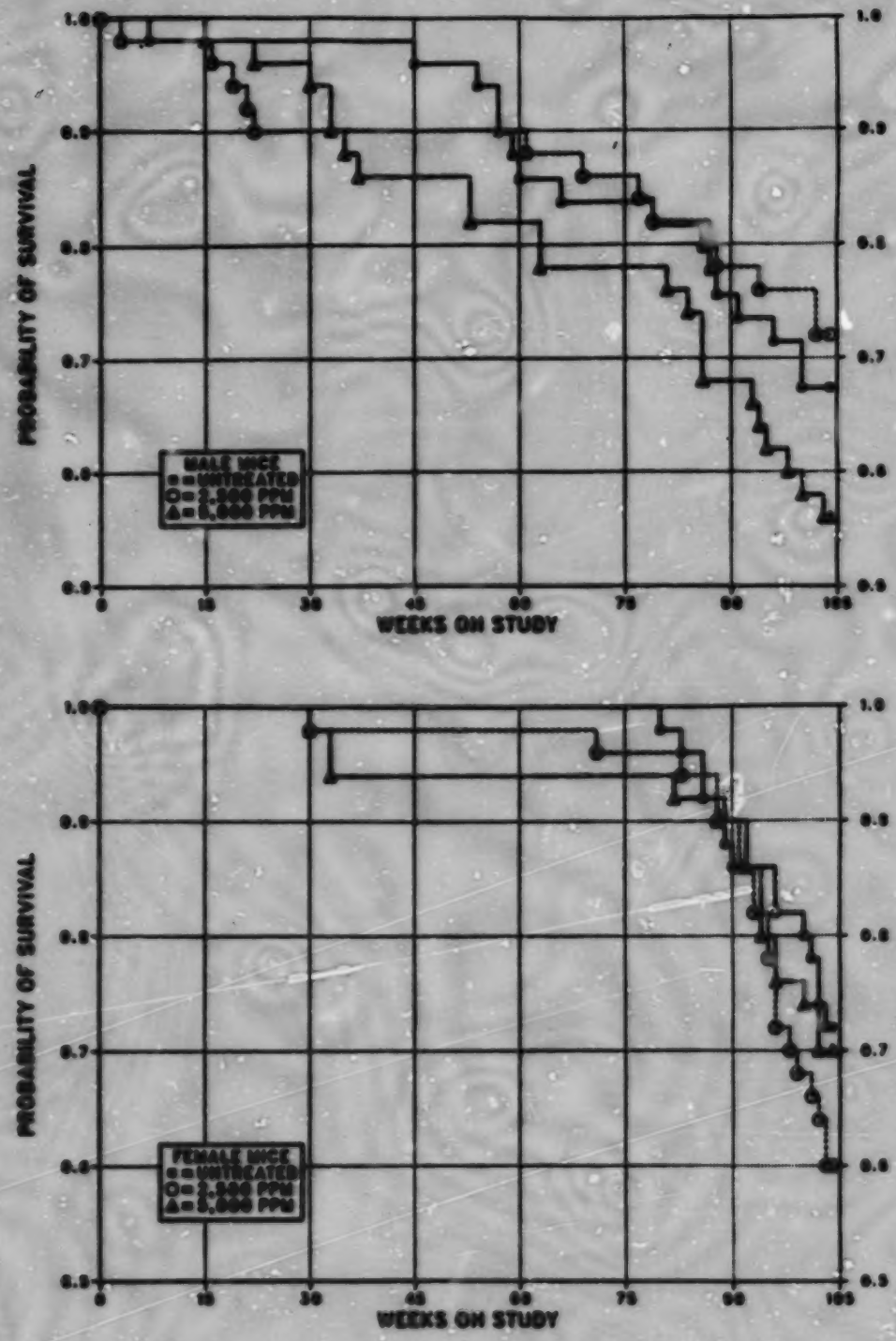

FIGURE 6. KAPLAN-MEIER SURVIVAL CURVES FOR MICE FED DIETS CONTAINING *N*-PHENYL-2-NAPHTHYLAMINE FOR TWO YEARS

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Kidney: Nuclear enlargement (karyomegaly) and minimal to mild nephropathy were observed at increased ($P<0.01$) incidences in high dose female mice (nuclear enlargement--female: control, 0/50; low dose, 0/50; high dose, 17/47; nephropathy--male: 30/49; 32/50; 31/47; female: 18/50; 16/50; 32/47). Nuclear enlargement was seen primarily in the convoluted tubules of the renal cortex; nephropathy consisted of a few scattered foci of tubular regeneration, thickened basement membranes, dilated tubules containing granular casts, and mononuclear cell infiltrates. Atypical tubular cell hyperplasia occurred in two high dose female mice. This lesion differed from the regenerative hyperplasia that is commonly part of nephropathy and exhibited cellular disorganization and slight cellular atypia. A tubular cell adenoma occurred in a third high dose female mouse, and a tubular cell adenocarcinoma occurred in a fourth high dose female.

Liver: Hepatocellular adenomas or carcinomas (combined) in male mice occurred with a positive trend by the life table test (control, 11/47; low dose, 16/50; high dose, 17/47; $P=0.046$); the incidences in the dosed groups were not significantly different from that in the controls. Hepatocellular adenomas or carcinomas (combined) were observed in 4/50 control, 3/14 low dose, and 7/48 high dose female mice.

Subcutaneous Tissue: Sarcomas, fibrosarcomas, or neurofibrosarcomas (combined) in male mice occurred with a significant positive trend; the incidence in the high dose group was significantly greater than that in the controls by life table tests (Table 18). The incidences of fibromas, sarcomas, fibrosarcomas, or neurofibrosarcomas (combined) in dosed male mice were not significantly different from that in the controls.

Ovary and Uterus: Suppurative inflammation or abscesses, primarily of the ovary and uterus but also of the fallopian tube, peritoneum, or multiple organs, were seen in 10/50 control, 15/50 low dose, and 19/50 high dose female mice.

TABLE 18. ANALYSIS OF SUBCUTANEOUS TISSUE TUMORS IN MALE MICE IN THE TWO-YEAR FEED STUDY OF *N*-PHENYL-2-NAPHTHYLAMINE (a)

	Control	2,500 ppm (b)	5,000 ppm (b)
Fibroma	2/49 (4%)	3/50 (6%)	0/50 (0%)
Sarcoma	0/49 (0%)	1/50 (2%)	5/50 (10%)
Neurofibrosarcoma	0/49 (0%)	1/50 (2%)	0/50 (0%)
Fibrosarcoma	2/49 (4%)	2/50 (4%)	3/50 (6%)
Sarcoma, Fibrosarcoma, or Neurofibrosarcoma			
Overall Rates	2/49 (4%)	4/50 (8%)	8/50 (16%)
Adjusted Rates	5.5%	9.6%	23.0%
Terminal Rates	1/33 (3%)	0/36 (0%)	3/28 (11%)
Week of First Observation	86	79	81
Life Table Tests	P=0.022	P=0.372	P=0.037
Incidental Tumor Tests	P=0.048	P=0.213	P=0.064
Fibroma, Sarcoma, Fibrosarcoma, or Neurofibrosarcoma (c)			
Overall Rates	4/49 (8%)	7/50 (14%)	8/50 (16%)
Adjusted Rates	11.0%	17.2%	23.0%
Terminal Rates	2/33 (6%)	3/36 (8%)	3/28 (11%)
Week of First Observation	86	79	81
Life Table Tests	P=0.107	P=0.314	P=0.137
Incidental Tumor Tests	P=0.216	P=0.193	P=0.247

(a) The statistical analyses used are discussed in Chapter II (Statistical Methods) and Appendix C, Table C3 (footnotes).
(b) The estimated dose in milligrams per kilograms per day is given in Chapter III (Body Weights and Clinical Signs) and in Appendix G.
(c) Historical incidence of fibromas, neurofibromas, sarcomas, fibrosarcomas, or neurofibrosarcomas (combined) at study laboratory (mean ± SD): 19/398 (5% ± 4%); historical incidence in NTP studies: 156/2,091 (7% ± 8%)

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IV. DISCUSSION AND CONCLUSIONS

Results of Short-Term Studies
Results of Two-Year Studies in Rats
Results of Two-Year Studies in Mice
Genotoxicity Studies
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IV. DISCUSSION AND CONCLUSIONS

N-Phenyl-2-naphthylamine, an antioxidant formerly used in the manufacture of rubber and plastics, was selected for toxicology and carcinogenesis studies because at the time of nomination (1976), it had a large annual production and widespread human exposure. Additional reasons for its selection included its structural similarity and possible metabolism to the known human urinary bladder carcinogen, 2-naphthylamine. The chemical used in the studies was 98% pure and contained less than 1 ppm 2-naphthylamine.

Studies of *N*-phenyl-2-naphthylamine were conducted in F344/N rats and B6C3F$_1$ mice for 14 days, 13 weeks, and 2 years. The compound was administered in the diet because *N*-phenyl-2-naphthylamine is stable in feed and dietary administration is a practical route of exposure.

Results of Short-Term Studies

In the 14-day studies, a dose-related decrease in the relative mean body weights of rats was observed. Male rats fed diets containing 50,000 ppm and female rats fed diets containing 25,000 or 50,000 ppm *N*-phenyl-2-naphthylamine lost weight. Deaths occurred in rats in the 50,000-ppm groups. The final mean body weights of surviving rats in these dosed groups were markedly lower than those of the controls for each sex. Arched backs, rough hair coats, and diarrhea were observed in males that received 12,500 ppm or more and in females that received 25,000 or 50,000 ppm. All mice survived to the end of the studies (dietary concentrations up to 20,000 ppm), and no compound-related clinical signs were observed.

In the 13-week studies, dose-related decreases in mean body weights were observed in rats; the final mean body weights of rats fed diets containing 10,000 ppm or more were lower than those of the controls. Compound-related deaths were seen in rats that received 40,000 ppm in the diet. Increases in liver weights and liver weight to body weight ratios relative to controls were observed for rats receiving 5,000 ppm (females only) or 10,000 ppm or more *N*-phenyl-2-naphthylamine in the diet. Nephropathy occurred at increased incidences in rats receiving 10,000 ppm (females only), 20,000 ppm, or 40,000 ppm. The lesion consisted of dilated tubules that contained reddish-brown granular material, remnants of tubular epithelial cells, and occasional degenerating leukocytes.

Dose-related decreases in mean body weight were observed in male and female mice. Male mice that received 40,000 ppm *N*-phenyl-2-naphthylamine in the diet lost weight. No compound-related clinical signs were observed in mice that survived to the end of the studies. Liver weights of male mice and liver weight to body weight ratios for male and female mice at 20,000 and 40,000 ppm were significantly greater than those for the controls. Dose-related increases in the incidences of nephropathy were observed in male and female mice. The lesions were characterized by necrosis of tubular epithelium, regeneration of tubular epithelial cells, and dilation of tubules, primarily in the renal cortex.

The increase in liver weights of rats and mice dosed with *N*-phenyl-2-naphthylamine may be due to the ability of this compound to induce microsomal enzymes. This speculation is supported by the finding that *N*-phenyl-2-naphthylamine is metabolized by the cytochrome P-450 system (Anderson et al., 1982) and by the ability of this compound to induce its own metabolism (Laham and Potvin, 1983).

Results of Two-Year Studies in Rats

Final mean body weights were lower than those of the controls for male rats at 5,000 ppm and for females at 2,500 and 5,000 ppm. Survival of rats was not adversely affected by administration of this compound. On the contrary, the number of dosed males and females surviving to the end of the studies was greater than that of the controls. The improved survival may simply be due to lower body weights of dosed rats. Dietary restriction resulting in decreased body weights is known to prolong the lifespan of animals (McCoy et al., 1935; Coneybeare, 1980). Ross (1966) estimated that for every 10% reduction in body weight, life expectancy increases 13.5%.

N-Phenyl-2-naphthylamine given in the diet to rats for 2 years increased the incidences of kidney lesions in both males and females. In dosed

female rats, there were increased incidences of kidney mineralization, necrosis of the renal papilla, kidney calculi, epithelial hyperplasia of the renal pelvis, chronic focal inflammation, hydronephrosis, atrophy, multifocal fibrosis, and acute suppurative inflammation. In dosed male rats, renal cysts and acute suppurative inflammation of the renal tubules were observed. This finding is consistent with the observation that aromatic amines have a high potential for producing kidney disease. Another chemical class with a high potential for producing chronic nephrotoxicity is the organohalides. Aromatic amines and organohalides account for more than 70% of the chemicals (45/62) found to cause renal injury in NTP/NCI studies (Kluwe et al., 1984).

A fibrosarcoma of the spleen was found in one low dose male rat, and a sarcoma of the spleen was found in one high dose male rat. Although no fibrosarcomas of the spleen were diagnosed in 1,954 untreated control male F344/N rats in NTP studies, these neoplasms may not be so rare. Sarcomas of the spleen were diagnosed in 5/1,954 (0.3%) male F344/N rats in NTP studies. These sarcomas represent anaplastic lesions similar to the fibrosarcomas seen in the low dose group. Additionally, the NTP guidelines for combining neoplasms in the evaluation of rodent carcinogenesis studies permit the combining of neoplasms of different morphologic classifications when the histomorphogenesis is comparable (McConnell et al., 1986). This combination makes the occurrence of one or two tumors of this type a less uncommon event. For these reasons, the fibrosarcoma and sarcoma observed here were not considered related to the administration of *N*-phenyl-2-naphthylamine. A fibrosarcoma was seen in the cecum of one low dose male rat and in the colon of a second low dose male rat. The historical incidences of fibrosarcomas and fibromas of the large intestine in untreated control male F344/N rats in NTP studies are 0/1,879 and 1/1,879 (0.05%). Because the two fibrosarcomas were observed in only the low dose group and because there is no evidence to indicate that the large intestine is a target organ for this chemical, the occurrence of these neoplasms was not considered to be related to *N*-phenyl-2-naphthylamine administration.

Leukemia and neoplasms of the thyroid, pituitary, and mammary glands were observed at decreased incidences in rats that received *N*-phenyl-2-naphthylamine in the diet (see Table [illegible]). The negative trends observed here may be related to the reduced body weights of rats receiving *N*-phenyl-2-naphthylamine (Roe, 1964; Tannenbaum, 1940). A decreased incidence of mammary gland fibroadenomas was previously found to be associated with lower weight gain in F344/N rats (Haseman, 1983). Correlations were found to exist between body weight and the incidences of leukemia, pituitary gland tumors, and mammary gland tumors in rats (Rao et al., 1987).

Disposition studies showed that dephenylation of *N*-phenyl-2-naphthylamine to 2-naphthylamine does not occur in male F344/N rats (SoRI, 1986). The absence of a carcinogenic effect of *N*-phenyl-2-naphthylamine in F344/N rats may be due to the inability of this strain to metabolize this compound to 2-naphthylamine. 2-Naphthylamine was not detected in an in vitro incubation mixture of rat liver microsomes and *N*-phenyl-2-naphthylamine (Anderson et al., 1982). These findings do not correspond with the in vivo studies of Laham and Potvin (1983) in which male Sprague Dawley rats did metabolize *N*-phenyl-2-naphthylamine to 2-naphthylamine.

Results of Two-Year Studies in Mice

Final mean body weights of dosed male and low dose female mice were comparable to those of the controls. The final mean body weight of the high dose females was 13% lower than that of the controls. Survival of dosed mice was not significantly different from that of the controls (see Table 17).

As in the rats, the primary organ affected was the kidney. Nuclear enlargement (karyomegaly) and minimal to mild nephropathy were observed at increased incidences in high dose female mice. Nuclear enlargement was seen primarily in the convoluted tubules of the renal cortex, and the nephropathy consisted of a few scattered foci of tubular regeneration, thickened basement membrane, dilated tubules containing

granular casts, and mononuclear cell infiltrates. Tubular cell hyperplasia was diagnosed in two high dose female mice. A tubular cell adenoma was diagnosed in a third high dose female mouse, and a tubular cell adenocarcinoma was diagnosed in a fourth high dose female mouse. The historical incidence of female B6C3F$_1$ mice with kidney tubular cell tumors is 0/394 at this laboratory and 1/2,079 (0.05%) throughout the Program. Kidney tumors are rare in female B6C3F$_1$ mice, but the evidence for an increased incidence of tumors in this study was considered marginal. Karyomegaly of tubular epithelial cells, a lesion that is sometimes associated with renal carcinogenesis, also occurred in female mice in this study.

Hepatocellular adenomas or carcinomas (combined) in male mice occurred with a marginally significant positive trend by the life table test (control, 11/47; low dose, 16/50; high dose, 17/47). Because the incidences in dosed male mice are similar to the historical rates at this laboratory (121/397, 30%) and throughout the Program (627/2,084, 30%) and because the positive trend observed for these neoplasms was significant by the life table test only, the increased incidence of liver neoplasms was considered to be unrelated to *N*-phenyl-2-naphthylamine administration. 2-Naphthylamine, a structurally related compound, caused increased incidences of liver tumors in mice (IARC, 1974).

Malignant mesenchymal tumors of the subcutaneous tissue (sarcomas, fibrosarcomas, or neurofibrosarcomas) occurred with a marginally significant positive trend. However, when these neoplasms are combined with subcutaneous tissue fibromas, the slightly elevated incidence is no longer statistically significant. Further, the incidence of these neoplasms (combined) is within the historical control range at this laboratory. For these reasons, the increased incidence of subcutaneous tissue neoplasms was considered to be unrelated to *N*-phenyl-2-naphthylamine administration. These neoplasms are combined for analysis because of their possible common origin from mesenchymal cells of the subcutis. Neoplasms classified as sarcomas are highly anaplastic (undifferentiated) neoplasms of undetermined histogenesis. Neurofibrosarcoma is diagnosed as such because of histologic similarity to human neoplasms that originate in the nerve sheath; the histogenesis in mice is uncertain. Suppurative inflammation or abscesses, primarily of the ovary and uterus but also of the fallopian tube, peritoneum, or multiple organs, were seen in 10/50 control, 15/50 low dose, and 19/50 high dose female mice.

Genotoxicity Studies

N-Phenyl-2-naphthylamine was not mutagenic in bacteria or in mammalian cells both with or without metabolic activation, nor did it induce chromosomal aberrations or SCEs in cultured mammalian cells. Its analog, *N*-phenyl-1-naphthylamine, also did not induce gene mutations in bacteria, yeast, or mammalian cells and did not induce chromosomal aberrations in cultured mammalian cells. However, weak positive responses with *N*-phenyl-1-naphthylamine have been reported for induction of unscheduled DNA synthesis in human cells (Brusick and Matheson, 1976) as well as for induction of SCEs in mammalian cells in the presence of S9 (NTP, unpublished results).

Data Audit

The experimental and tabulated data for the NTP Technical Report on *N*-phenyl-2-naphthylamine were examined for accuracy, consistency, completeness, and compliance with Good Laboratory Practice regulations. As summarized in Appendix I, the audit revealed no problems with the conduct of the studies or with collection and documentation of the experimental data. No discrepancies were found that influenced the final interpretation of the results of these studies.

Conclusions

*U*nder the conditions of these 2-year feed studies, there was *no evidence of carcinogenic activity*[a] for male or female F344/N rats fed diets containing 2,500 or 5,000 ppm *N*-phenyl-2-naphthylamine. Decreased incidences of several neoplasms were observed in dosed rats: thyroid gland C-cell neoplasms in males and females and mononuclear cell leukemia, pituitary gland adenomas, and mammary gland fibroadenomas in females. There was *no evidence of carcinogenic activity* for male $B6C3F_1$ mice fed diets containing 2,500 or 5,000 ppm *N*-phenyl-2-naphthylamine. There was *equivocal evidence of carcinogenic activity* of *N*-phenyl-2-naphthylamine for female $B6C3F_1$ mice as indicated by the occurrence of two rare kidney neoplasms. Chemical-related nonneoplastic lesions (nephropathy, karyomegaly, and hyperplasia) occurred in the kidney of rats and mice.

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[a]Explanation of Levels of Evidence of Carcinogenic Activity is on page 7.
A summary of the Peer Review comments and the public discussion on this Technical Report appears on page 11.

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V. REFERENCES

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1. Althaus, F.R.; Lawrence, S.D.; Sattler, G.L.; Longfellow, D.G.; Pitot, H.C. (1982) Chemical quantification of unscheduled DNA synthesis in cultured hepatocytes as an assay for the rapid screening of potential chemical carcinogens. Cancer Res. 42:3010-3015.

2. Anderson, D.; Styles, J.A. (1978) Appendix II. The bacterial mutagenicity test. Purchase, I.F.H.; et al., Eds.: Six Tests for Carcinogenicity. Br. J. Cancer 37:924-930.

3. Anderson, M.M.; Mitchum, R.K.; Beland, F.A. (1982) Hepatic microsomal metabolism and macromolecular binding of the antioxidant, *N*-phenyl-2-naphthylamine. Xenobiotica 12:31-43.

4. Armitage, P. (1971) Statistical Methods in Medical Research. New York: John Wiley & Sons Inc., pp. 362-365.

5. Bartsch, H.; Malaveille, C.; Camus, A.-M.; Martel-Planche, G.; Brun, A.; Hautefeuille, N.; Sabadie, A.; Barbin, T.; Kuroki, T.; Drevon, C.; Piccoli, C. (1980) Validation and comparative studies on 180 chemicals with *S. typhimurium* strains and V79 Chinese hamster cells in the presence of various metabolizing systems. Mutat. Res. 76:1-50.

6. Batten, P.L.; Hathway, D.E. (1977) Dephenylation of *N*-phenyl-2-naphthylamine in dogs and its possible oncogenic implications. Br. J. Cancer 35:342-346.

7. Berenblum, I., Ed. (1969) Carcinogenicity Testing: A Report of the Panel on Carcinogenicity of the Cancer Research Commission of UICC, Vol. 2. Geneva: International Union Against Cancer.

8. Boorman, G.A.; Montgomery, C.A., Jr.; Eustis, S.L.; Wolfe, M.J.; McConnell, E.E.; Hardisty, J.F. (1985) Quality assurance in pathology for rodent carcinogenicity studies. Milman, H.; Weisburger, E., Eds.: Handbook of Carcinogen Testing. Park Ridge, NJ: Noyes Publications, pp. 345-357.

9. Braden, J.M.; Kelley, M.; Simmon, V.F.; Rice, S.A.; Mazze, R.I. (1978) Fluroxene mutagenicity. Mutat. Res. 58:183-191.

10. Brusick, D.; Matheson, D.W. (1976) Mutagen and Oncogen Study on N-Phenyl-alpha-naphthylamine. U.S. NTIS Ad. Report (ADA035476).

11. Conneybeare, G. (1980) Effect of quality and quantity of diet on survival and tumor incidence in outbred Swiss mice. Food Cosmet. Toxicol. 18:65-76.

12. Cox, D.R. (1972) Regression models and life tables. J. R. Stat. Soc. B34:187-220.

13. Dunkel, V.C.; Zeiger, E.; Brusick, D.; McCoy, E.; McGregor, D.; Mortelmans, K.; Rosenkranz, H.S.; Simmon, V. (1984) Reproducibility of microbial mutagenicity assays. I. Tests with *Salmonella typhimurium* and *Escherichia coli* using a standardized protocol. Environ. Mutagen. 6(Suppl. 2):1-251.

14. Dunnett, C.W. (1955) A multiple comparison procedure for comparing several treatments with a control. J. Am. Stat. Assoc. 50:1096-1122.

15. Fox, A.J.; Collier, P.F. (1976) A survey of occupational cancer in the rubber and cable-making industries: Analysis of deaths occurring in 1972-74. Br. J. Ind. Med. 33:249-264.

16. Galloway, S.M.; Bloom, A.D.; Resnick, M.; Margolin, B.H.; Nakamura, F.; Archer, P.; Zeiger, E. (1985) Development of a standard protocol for in vitro cytogenetic testing with Chinese hamster ovary cells: Comparison of results for 22 compounds in two laboratories. Environ. Mutagen. 7:1-51.

17. Gart, J.J.; Chu, K.C.; Tarone, R.E. (1979) Statistical issues in interpretation of chronic bioassay tests for carcinogenicity. J. Natl. Cancer Inst. 62:957-974.

18. Gehrmann, G.H.; Foulger, J.H.; Fleming, A.J. (1949) Occupational tumours of the bladder. Proceedings of the 9th International Congress of Industrial Medicine, London, 1948. Bristol, England: Wright, pp. 472-475.

19. Green, U.; Holste, J.; Spikermann, A.R. (1979) A comparative study of the chronic effects of magenta, paramagenta, and phenyl-β-naphthylamine in Syrian golden hamsters. J. Cancer Res. Clin. Oncol. 95:51-55.

20. Gupta, R.S.; Goldstein, S. (1981) Mutagen testing in the human fibroblast diphtheria toxin resistance (HR DIPR) system. Evaluation of Short-Term Tests for Carcinogens: Report of the International Collaborative Program. Prog. Mutat. Res. 1:614-625.

21. Haseman, J.K. (1983) Patterns of tumor incidence in two-year cancer bioassay feeding studies in Fischer 344 rats. Fundam. Appl. Toxicol. 3:1-9.

22. Haseman, J.K. (1984) Statistical issues in the design, analysis and interpretation of animal carcinogenicity studies. Environ. Health Perspect. 58:385-392.

23. Haseman, J.K.; Huff, J.; Boorman, G.A. (1984) Use of historical control data in carcinogenicity studies in rodents. Toxicol. Pathol. 12:126-135.

24. Haseman, J.K.; Huff, J.; Rao, G.N.; Arnold, J.; Boorman, G.A.; McConnell, E.E. (1985) Neoplasms observed in untreated and corn oil gavage control groups of F344/N rats and (C57BL/6N × C3H/HeN)F_1 ($B6C3F_1$) mice. J. Natl. Cancer Inst. 75:975-984.

25. Haworth, S.; Lawlor, T.; Mortelmans, K.; Speck, W.; Zeiger, E. (1983) Salmonella mutagenicity test results for 250 chemicals. Environ. Mutagen. Suppl. 1:3-142.

26. Innes, J.R.M.; Ulland, B.M.; Valerio, M.G.; Petrucelli, L.; Fishbein, L.; Hart, E.R.; Pallotta, A.J.; Bates, R.R.; Falk, H.L.; Gart, J.J.; Klein, M.; Mitchell, I.; Peters, J. (1969) Bioassay of pesticides and industrial chemicals for tumorigenicity in mice: A preliminary note. J. Natl. Cancer Inst. 42:1101-1114.

27. International Agency for Research on Cancer (IARC) (1974) 2-Naphthylamine. Some Aromatic Amines, Hydrazine and Related Substances, N-Nitroso Compounds and Miscellaneous Alkylating Agents. IARC Monographs on the Evaluation of the Carcinogenic Risks of Chemicals to Man, Vol. 4. Lyon, France: IARC, pp. 97-111.

28. International Agency for Research on Cancer (IARC) (1978) *N*-Phenyl-2-naphthylamine. Some Aromatic Amines and Related Nitro Compounds--Hair Dyes, Colouring Agents, and Miscellaneous Industrial Chemicals. IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Man, Vol. 16. Lyon, France: IARC, pp. 325-341.

29. Kaplan, E.L.; Meier, P. (1958) Nonparametric estimation from incomplete observations. J. Am. Stat. Assoc. 53:457-481.

30. Kawachi, T.; Komatsu, T.; Kada, M.; Ishidate, M.; Sasaki, T.; Sugiyama, T.; Tazima, Y. (1980a) Results of recent studies on the relevance of various short-term screening tests in Japan. The Predictive Value of Short-Term Screening Tests in Carcinogenicity Evaluation. Appl. Methods Oncol. 3:253-267.

31. Kawachi, T.; Yahagi, T.; Kada, T.; Tazima, T.; Ishidate, M.; Sasaki, M.; Sugiyama, T. (1980b) Cooperative program on short-term assays for carcinogenicity in Japan. IARC Sci. Publ. 27:323-330.

32. Kehe, H.J.; Kouris, C.S. (1965) Diarylamines. Kirk, R.E.; Othmer, D.F., Eds.: Encyclopedia of Chemical Technology, Vol. 7, 2nd ed. New York: John Wiley & Sons Inc., pp. 40-49.

33. Ketkar, M.B.; Mohr, U.B. (1982) The chronic effects of magenta, paramagenta and phenyl-β-naphthylamine in rats after intragastric administration. Cancer Lett. 16:203-206.

34. Kirkhart, B. (1981) Micronucleus test on 21 compounds. Evaluation of Short-Term Tests for Carcinogens: Report of the International Collaborative Program. Prog. Mutat. Res. 1:698-704.

35. Kluwe, W.M.; Abdo, K.M.; Huff, J. (1984) Chronic kidney disease and organic chemical exposures: Evaluations of causal relationships in humans and experimental animals. Fundam. Appl. Toxicol. 4:889-901.

V. REFERENCES

36. Kummer, R.; Tordoir, W.F. (1975) Phenylbetanaphthylamine (PBNA), another carcinogenic agent? T. Soc. Geneesk. 53:415-419.

37. Laham, S.; Potvin, M. (1983) Biological conversion of N-phenyl-2-naphthylamine in the Sprague-Dawley rat. Drug Chem. Toxicol. 6:295-309.

38. Linhart, M.S.; Cooper, J.; Martin, R.L.; Page, N.; Peters, J. (1974) Carcinogenesis Bioassay Data System. Comput. Biomed. Res. 7:230-248.

39. Mantel, N.; Haenszel, W. (1959) Statistical aspects of the analysis of data from retrospective studies of disease. J. Natl. Cancer Inst. 22:719-748.

40. Maronpot, R.R.; Boorman, G.A. (1982) Interpretation of rodent hepatocellular proliferative alterations and hepatocellular tumors in chemical safety assessment. Toxicol. Pathol. 10:71-80.

41. McConnell, E.E. (1983a) Pathology requirements for rodent two-year studies. I. A review of current procedures. Toxicol. Pathol. 11:60-64.

42. McConnell, E.E. (1983b) Pathology requirements for rodent two-year studies. II. Alternative approaches. Toxicol. Pathol. 11:65-76.

43. McConnell, E.E.; Solleveld, H.A.; Swenberg, J.A.; Boorman, G.A. (1986) Guidelines for combining neoplasms for evaluation of rodent carcinogenesis studies. J. Natl. Cancer Inst. 76:283-289.

44. McCoy, C.M.; Crowell, M.E.; Maynard, L.A. (1935) The effect of retarded growth upon the length of life span and ultimate size. J. Nutr. 10:63-79.

45. Moore, R.M., Jr.; Woolf, B.S.; Stein, H.P.; Thomas, A.W.; Finklea, J.F. (1977) Metabolic precursors of a known human carcinogen. Science 195:344.

46. Natarajan, A.T.; Van Kesteren-Van Leeuwen, A.C. (1981) Mutagenic activity of 20 coded compounds in chromosome aberrations/sister chromatid exchanges assay using Chinese hamster ovary (CHO) cells. Evaluation of Short-Term Tests for Carcinogens: Report of the International Collaborative Program. Prog. Mutat. Res. 1:551-559.

47. National Cancer Institute (NCI) (1976) Guidelines for Carcinogen Bioassay in Small Rodents. NCI Technical Report No. 1. U.S. Department of Health, Education, and Welfare, Public Health Service, National Institutes of Health, Bethesda, MD.

48. National Cancer Institute (NCI) (1977) Frequency of Organic Compounds Identified in Water. Summary of Data for Chemical Selection. Submitted by U.S. Environmental Protection Agency.

49. National Institute for Occupational Safety and Health (NIOSH) (1977) Current NIOSH intelligence bulletin: Metabolic precursors of a known carcinogen, beta-naphthylamine. Am. Ind. Hyg. Assoc. J. 38:A21-A23.

50. National Institutes of Health (NIH) (1978) Open Formula Rat and Mouse Ration (NIH-07). Specification NIH-11-1335. U.S. Department of Health, Education, and Welfare, Public Health Service, National Institutes of Health, Bethesda, MD.

51. Parodi, S.; Zunino, A.; Ottagio, L.; De Ferrari, M.; Santi, L. (1983) Lack of correlation between the capability of inducing sister chromatid exchanges in vivo and carcinogenic potency, for 16 aromatic amines and azo derivatives. Mutat. Res. 108:225-238.

52. Rao, G.N.; Piegorsch, W.W.; Haseman, J.K. (1987) Influence of body weight on the incidence of spontaneous tumors in rats and mice of long-term studies. Am. J. Clin. Nutr. 45:252-260.

53. Roe, F.J.C. (1984) Perspectives in carbohydrate toxicology with special reference to carcinogenicity. Swed. Dent. J. 8:99-111.

54. Ross, M.H. (1966) Life expectancy modification by change in dietary regimen of the mature rat. Kuhnam, J., Ed.: Proc. 7th Intern. Congr. Nutr., Vol. 5. New York: Pergamon Press, p. 35.

55. Sax, N.I., Ed. (1984) Dangerous Properties of Industrial Materials, 6th ed. New York: Van Nostrand Reinhold Company, p. 2196.

56. Sharma, G.P.; Sobti, R.C.; Sahi, K. (1980) Mutagenic effects of some aromatic amines on rat chromosomes. Nucleus (Calcutta) 23:36-46.

57. Southern Research Institute (SoRI) (1986) Metabolism of *N*-phenyl-2-naphthylamine After Feeding to Fischer 344 Rats. SoRI-86-984. Prepared for the National Institute of Environmental Health Sciences, Research Triangle Park, NC. 8 p.

58. Tannenbaum, A. (1940) The initiation and growth of tumors. I. Effect of underfeeding. Am. J. Cancer 38:335-350.

59. Tarone, R.E. (1975) Tests for trend in life table analysis. Biometrika 62:679-682.

60. U.S. International Trade Commission (USITC) (1975) Synthetic Organic Chemicals, U.S. Production and Sales, 1973. USITC Publication 728. Washington, DC: U.S. Government Printing Office, pp. 136, 139.

61. U.S. International Trade Commission (USITC) (1976) Synthetic Organic Chemicals, U.S. Production and Sales, 1974. USITC Publication 776. Washington, DC: U.S. Government Printing Office, pp. 42, 136, 139.

62. U.S. International Trade Commission (USITC) (1977) Synthetic Organic Chemicals, U.S. Production and Sales, 1975. USITC Publication 804. Washington, DC: U.S. Government Printing Office, pp. 133, 134.

63. Vogel, E.W.; Zijlstra, J.A.; Blijleven, W.G.H. (1983) Mutagenic activity of selected aromatic amines and polycyclic hydrocarbons in *Drosophila melanogaster*. Mutat. Res. 107:53-77.

64. Wang, C.Y.; Garner, C.D.; Lee, M.S.; Shirai, T. (1981) *O*-Esters of *N*-acylhydroxylamines: Toxicity and enhancement of sister-chromatid exchange in Chinese hamster ovary cells. Mutat. Res. 88:81-88.

65. Wang, H.-W.; You, X.; Qu, Y.-H.; Wang, W.-F.; Wang, D.; Long, Y.-M.; Ni, J.-A. (1984a) Investigation of cancer epidemiology and study of carcinogenic agents in the Shanghai rubber industry. Cancer Res. 44:3101-3105.

66. Wang, H.-W.; Wang, D.; Dzeng, R.-W. (1984b) Carcinogenicity of *N*-phenyl-1-naphthylamine and *N*-phenyl-2-naphthylamine in mice. Cancer Res. 44:3098-3100.

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APPENDIX A

SUMMARY OF LESIONS IN MALE RATS IN THE TWO-YEAR FEED STUDY OF *N*-PHENYL-2-NAPHTHYLAMINE

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TABLE A1. SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MALE RATS IN THE TWO-YEAR FEED STUDY OF *N*-PHENYL-2-NAPHTHYLAMINE

	Untreated Control	Low Dose	High Dose
ANIMALS INITIALLY IN STUDY	50	50	50
ANIMALS NECROPSIED	50	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY	50	50	50
INTEGUMENTARY SYSTEM			
*Skin	(50)	(50)	(50)
Squamous cell carcinoma			1 (2%)
Trichoepithelioma	1 (2%)	1 (2%)	
Keratoacanthoma	†4 (8%)	1 (2%)	
*Subcutaneous tissue	(50)	(50)	(50)
Fibroma	1 (2%)		1 (2%)
Fibrosarcoma	1 (2%)		
Fibrous histiocytoma, malignant		1 (2%)	
RESPIRATORY SYSTEM			
#Lung	(50)	(9)	(49)
Squamous cell carcinoma, metastatic	1 (2%)		
Alveolar/bronchiolar adenoma	1 (2%)		4 (8%)
Alveolar/bronchiolar carcinoma	3 (6%)		
C-cell carcinoma, metastatic	1 (2%)		
Pheochromocytoma, metastatic		1 (11%)	
Fibrous histiocytoma, metastatic		1 (11%)	
HEMATOPOIETIC SYSTEM			
*Multiple organs	(50)	(50)	(50)
Leukemia, mononuclear cell	21 (42%)	29 (58%)	24 (48%)
#Spleen	(49)	(30)	(49)
Sarcoma, NOS			1 (2%)
Fibrosarcoma		1 (3%)	
#Mandibular lymph node	(47)	(15)	(48)
C-cell carcinoma, metastatic	1 (2%)		
#Mediastinal lymph node	(47)	(15)	(48)
Squamous cell carcinoma, metastatic	1 (2%)		
#Pancreatic lymph node	(47)	(15)	(48)
Fibrous histiocytoma, metastatic		1 (7%)	
CIRCULATORY SYSTEM			
None			
DIGESTIVE SYSTEM			
*Tongue	(50)	(50)	(50)
Squamous cell papilloma		1 (2%)	
#Liver	(50)	(50)	(50)
Neoplastic nodule		3 (6%)	
Hepatocellular carcinoma	2 (4%)		
Fibrous histiocytoma, metastatic		1 (2%)	
#Pancreas	(46)	(9)	(50)
Acinar cell adenoma	2 (4%)		1 (2%)
#Forestomach	(49)	(7)	(49)
Squamous cell papilloma		1 (14%)	
Squamous cell carcinoma	1 (2%)		
#Colon	(48)	(8)	(48)
Fibrosarcoma		1 (13%)	
#Cecum	(48)	(8)	(48)
Fibrosarcoma		1 (13%)	

TABLE A1. SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MALE RATS IN THE TWO-YEAR FEED STUDY OF *N*-PHENYL-2-NAPHTHYLAMINE (Continued)

	Untreated Control	Low Dose	High Dose
URINARY SYSTEM			
#Kidney	(50)	(50)	(50)
Tubular cell adenoma	2 (4%)		
Tubular adenocarcinoma	1 (2%)		
#Kidney/capsule	(50)	(50)	(50)
Mesothelioma, NOS		1 (2%)	
#Kidney/cortex	(50)	(50)	(50)
Tubular cell adenoma			1 (2%)
ENDOCRINE SYSTEM			
#Pituitary	(49)	(12)	(49)
Neurofibrosarcoma	1 (2%)		
#Anterior pituitary	(49)	(12)	(49)
Carcinoma, NOS	1 (2%)		
Adenoma, NOS	14 (29%)	5 (42%)	7 (14%)
#Adrenal	(50)	(10)	(50)
Cortical adenoma	1 (2%)		1 (2%)
#Adrenal medulla	(50)	(10)	(50)
Pheochromocytoma	12 (24%)	4 (40%)	20 (40%)
Pheochromocytoma, malignant		2 (20%)	
Ganglioneuroma	1 (2%)		
#Periadrenal tissue	(50)	(10)	(50)
Fibroma	1 (2%)		
#Thyroid	(49)	(50)	(49)
Follicular cell adenoma	1 (2%)		1 (2%)
Follicular cell carcinoma			1 (2%)
C-cell adenoma	7 (14%)	7 (14%)	4 (8%)
C-cell carcinoma	2 (4%)		
#Parathyroid	(39)	(43)	(43)
Adenoma, NOS			1 (2%)
#Pancreatic islets	(46)	(9)	(50)
Islet cell adenoma	1 (2%)		
REPRODUCTIVE SYSTEM			
*Mammary gland	(50)	(50)	(50)
Fibroadenoma		1 (2%)	
*Prepuce	(50)	(50)	(50)
Squamous cell carcinoma			1 (2%)
*Preputial gland	(50)	(50)	(50)
Carcinoma, NOS	1 (2%)	1 (2%)	
Adenoma, NOS	1 (2%)		1 (2%)
#Testis	(50)	(50)	(49)
Interstitial cell tumor	43 (86%)	49 (98%)	47 (96%)
NERVOUS SYSTEM			
#Brain/meninges	(50)	(6)	(50)
Neurofibrosarcoma, invasive	1 (2%)		
#Cerebrum	(50)	(6)	(50)
Oligodendroglioma	1 (2%)		
#Brain	(50)	(6)	(50)
Meningioma	1 (2%)		
#Cerebellum	(50)	(6)	(50)
Astrocytoma	1 (2%)		

TABLE A1. SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MALE RATS IN THE TWO-YEAR FEED STUDY OF *N*-PHENYL-2-NAPHTHYLAMINE (Continued)

	Untreated Control	Low Dose	High Dose
SPECIAL SENSE ORGANS			
*Nasolacrimal duct	(50)	(50)	(50)
Squamous cell carcinoma	1 (2%)		
*Zymbal gland	(50)	(50)	(50)
Carcinoma, NOS	1 (2%)		1 (2%)
MUSCULOSKELETAL SYSTEM			
*Cervical vertebra other	(50)	(50)	(50)
Osteosarcoma	1 (2%)		
BODY CAVITIES			
*Mediastinum	(50)	(50)	(50)
Alveolar/bronchiolar carcinoma, invasive	2 (4%)		
C-cell carcinoma, invasive	1 (2%)		
Mesothelioma, NOS	1 (2%)		
*Abdominal cavity	(50)	(50)	(50)
Squamous cell carcinoma, invasive	1 (2%)		
*Parietal peritoneum	(50)	(50)	(50)
Mesothelioma, metastatic			1 (2%)
*Visceral peritoneum	(50)	(50)	(50)
Mesothelioma, metastatic			1 (2%)
*Pericardium	(50)	(50)	(50)
Alveolar/bronchiolar carcinoma, invasive	1 (2%)		
*Epicardium	(50)	(50)	(50)
Alveolar/bronchiolar carcinoma, invasive	1 (2%)		
*Mesentery	(50)	(50)	(50)
Mesothelioma, NOS		2 (4%)	
*Tunica vaginalis	(50)	(50)	(50)
Mesothelioma, NOS	2 (4%)	2 (4%)	
Mesothelioma, malignant			2 (4%)
ALL OTHER SYSTEMS			
*Multiple organs	(50)	(50)	(50)
Mesothelioma, NOS		1 (2%)	
Mesothelioma, metastatic			1 (2%)
ANIMAL DISPOSITION SUMMARY			
Animals initially in study	50	50	50
Natural death	6	8	7
Moribund sacrifice	20	14	9
Terminal sacrifice	24	28	34

TABLE A1. SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MALE RATS IN THE TWO-YEAR FEED STUDY OF *N*-PHENYL-2-NAPHTHYLAMINE (Continued)

	Untreated Control	Low Dose	High Dose
TUMOR SUMMARY			
Total animals with primary tumors**	50	50	50
Total primary tumors	127	116	130
Total animals with benign tumors	47	48	48
Total benign tumors	94	79	89
Total animals with malignant tumors	36	34	39
Total malignant tumors	40	36	41
Total animals with secondary tumors##	5	2	2
Total secondary tumors	11	4	3
Total animals with tumors uncertain--benign or malignant	3	5	
Total uncertain tumors	3	9	

* Number of animals receiving complete necropsy examinations; all gross lesions (including masses) examined microscopically
† Multiple occurrence of morphology in the same organ. Tissue is counted once only.
** Primary tumors: all tumors except secondary tumors
# Number of animals examined microscopically at this site
Secondary tumors: metastatic tumors or tumors invasive into an adjacent organ

TABLE A5. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF MALE RATS IN THE TWO-YEAR FEED STUDY OF *N*-PHENYL-2-NAPHTHYLAMINE: UNTREATED CONTROL

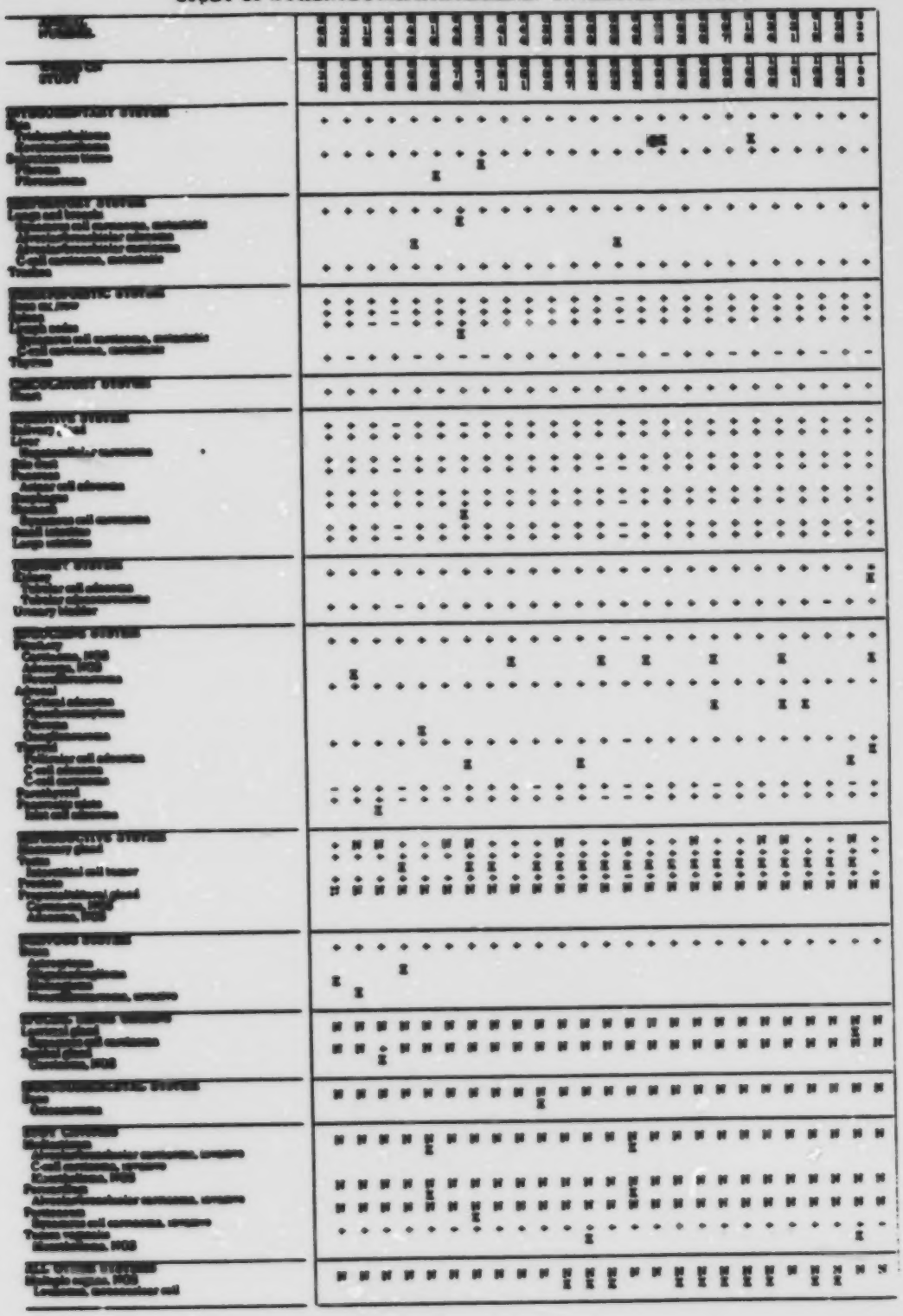
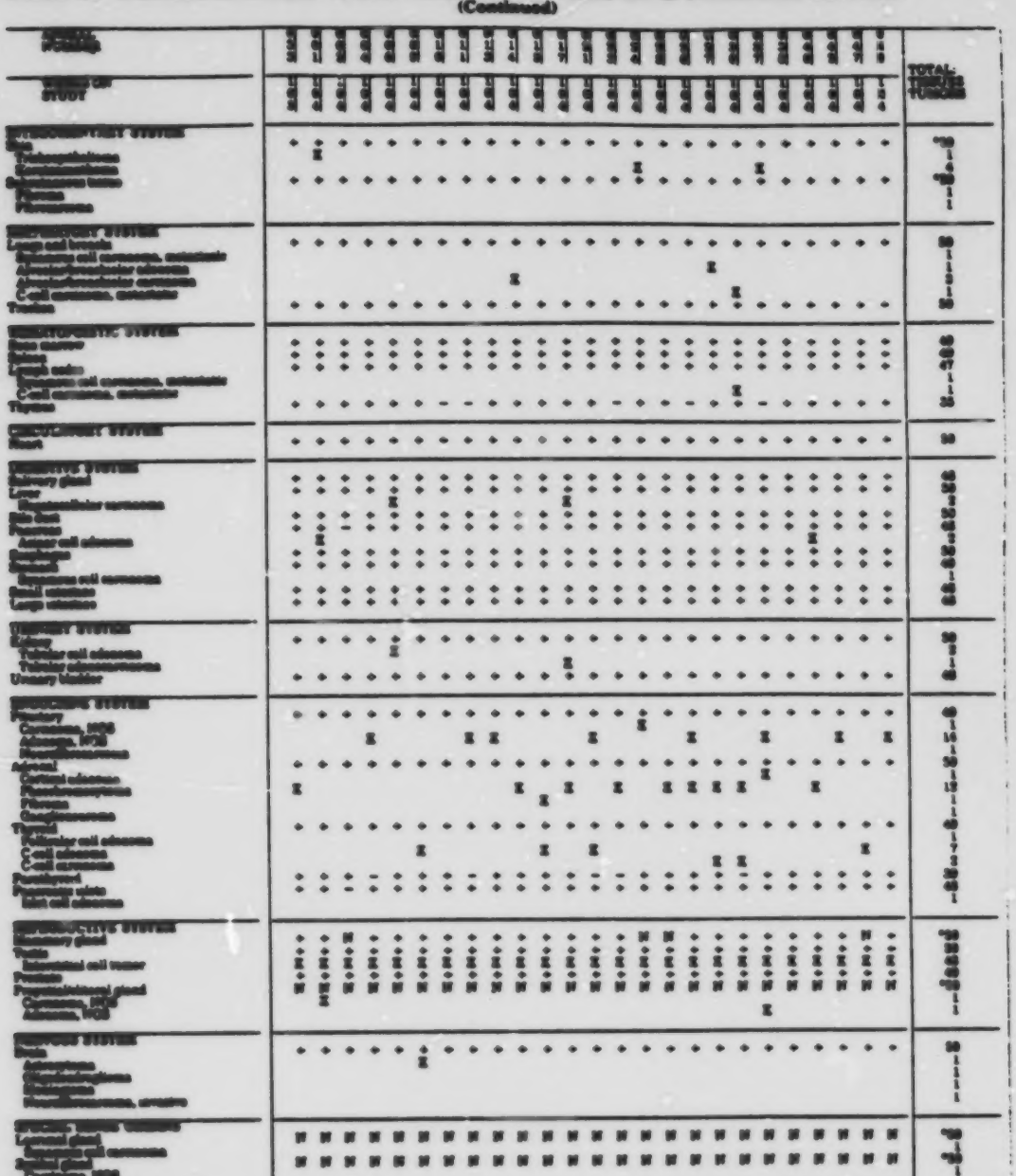


TABLE A3. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF MALE RATS: UNTREATED CONTROL (Continued)



* Animals necropsied

TABLE A2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF MALE RATS IN THE TWO-YEAR FEED STUDY OF *N*-PHENYL-2-NAPHTHYLAMINE: LOW DOSE

ANIMAL NUMBER	0 2 3	0 2 6	0 2 1	0 2 2	0 2 4	0 2 5	0 2 8	0 2 9	0 3 0	0 3 1	0 3 2	0 3 3	0 3 4	0 3 5	0 3 6	0 3 7	0 3 8	0 3 9	0 4 0	0 4 1	0 4 2	0 4 3	0 4 4	0 4 5	0 4 6	0 4 7	0 4 8	0 4 9	0 5 0	0 5 3
WEEKS ON STUDY	0 2 3	0 3 6	0 3 1	0 3 6	0 3 6	0 3 6	0 3 6	0 3 6	0 3 6	0 3 6	0 3 6	0 3 6	0 3 6	0 3 6	0 3 6	0 3 6	0 3 6	0 3 6	0 3 6	0 3 6	0 3 6	0 3 6	0 3 6	0 3 6	0 3 6	0 3 6	0 3 6	0 3 6	0 3 6	0 4 4
INTEGUMENTARY SYSTEM																														
Skin	+	+	+	+	+	+	M	M	M	M	M	M	+	M	M	+	M	M	M	M	M	+	M	M	M					
Trichoepithelioma																X														
Keratoacanthoma																														
Subcutaneous tissue	+	+	+	+	+	+	M	M	M	M	M	M	+	M	M	+	M	M	M	M	M	+	M	M	M					
Fibrous histiocytoma, malignant																						X								
RESPIRATORY SYSTEM																														
Lungs and bronchi	+	+	+	+	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+	+	–	–					
Pheochromocytoma, metastatic	X																													
Fibrous histiocytoma, metastatic																						X								
Trachea	+	+	+	+	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–					
HEMATOPOIETIC SYSTEM																														
Bone marrow	+	+	+	+	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–					
Spleen	+	+	+	+	+	+	+	+	+	+	–	–	–	+	–	+	+	–	–	+	+	–	+	–	+					
Fibrosarcoma																														
Lymph nodes	+	+	+	+	+	+	–	+	–	+	–	–	+	–	+	–	–	–	+	+	+	+	–	–	–					
Fibrous histiocytoma, metastatic																						X								
Thymus	+	+	+	+	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–					
CIRCULATORY SYSTEM																														
Heart	+	+	+	+	+	+	–	–	–	–	–	+	–	–	–	–	–	+	+	–	–	+	–	–	–					
DIGESTIVE SYSTEM																														
Oral cavity	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M					
Squamous cell papilloma																														
Salivary gland	+	+	+	+	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–					
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+					
Neoplastic nodule																						X								
Fibrous histiocytoma, metastatic																														
Bile duct	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+					
Pancreas	+	+	+	+	+	+	–	–	–	+	–	–	–	–	–	–	–	+	+	–	–	–	–	–	–					
Esophagus	+	+	+	+	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–					
Stomach	–	+	+	+	+	+	–	–	–	–	–	–	+	–	–	+	–	–	–	–	–	–	–	–	–					
Squamous cell papilloma																X														
Small intestine	–	+	–	+	+	+	–	–	–	–	–	–	–	–	–	+	–	–	+	–	+	–	–	–	–					
Large intestine	+	+	+	+	+	–	–	–	–	–	–	–	–	–	–	+	–	–	–	–	+	–	–	–	–					
Fibrosarcoma																					X									
URINARY SYSTEM																														
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+					
Mesothelioma, NOS																X														
Urinary bladder	+	+	+	+	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–					
ENDOCRINE SYSTEM																														
Pituitary	+	+	+	+	+	+	–	–	–	–	–	+	+	–	–	–	–	–	–	–	–	+	–	–	–					
Adenoma, NOS												X	X									X								
Adrenal	+	+	+	+	+	+	–	–	–	–	–	+	–	–	–	–	–	+	–	–	–	–	–	–	–					
Pheochromocytoma												X						X												
Pheochromocytoma, malignant	X																	X												
Thyroid	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+					
C-cell adenoma						X																								
Parathyroid	+	–	+	–	+	+	+	+	–	+	+	+	+	+	–	+	+	+	+	+	–	+	+	+	+					
REPRODUCTIVE SYSTEM																														
Mammary gland	M	M	+	+	+	M	M	+	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M					
Fibroadenoma																														
Testis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+					
Interstitial cell tumor	X	X	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X					
Prostate	+	+	+	+	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–					
Preputial/clitoral gland	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M					
Carcinoma, NOS																														
NERVOUS SYSTEM																														
Brain	+	+	+	+	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–					
BODY CAVITIES																														
Tunica vaginalis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+					
Mesothelioma, NOS																X														
Mesentery	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M					
Mesothelioma, NOS																X														
ALL OTHER SYSTEMS																														
Multiple organs, NOS	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M					
Mesothelioma, NOS		X																												
Leukemia, mononuclear cell				X	X	X	X	X	X	X		X		X	X		X	X		Y			X	X						

TABLE A2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF MALE RATS: LOW DOSE
(Continued)

ANIMAL NUMBER	0 0 4	0 0 5	0 0 6	0 0 7	0 0 8	0 0 9	0 1 0	0 1 1	0 1 2	0 1 3	0 1 4	0 1 5	0 1 6	0 1 7	0 1 8	0 1 9	0 2 0	0 2 1	0 2 2	0 2 3	0 2 4	0 2 5	0 2 6	0 2 7	0 2 8	0 2 9	0 3 0	TOTAL: TISSUES TUMORS
WEEKS ON STUDY	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	
INTEGUMENTARY SYSTEM																												
Skin	N	N	N	N	N	N	N	N	N	N	N	+	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	*50
Trichoepithelioma												X																1
Keratoacanthoma																												1
Subcutaneous tissue	N	N	N	N	N	N	N	N	N	N	N	+	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	*50
Fibrous histiocytoma, malignant																												1
RESPIRATORY SYSTEM																												
Lungs and bronchi	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	9
Pheochromocytoma, metastatic																												1
Fibrous histiocytoma, metastatic																												1
Trachea	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	6
HEMATOPOIETIC SYSTEM																												
Bone marrow	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	6
Spleen	-	-	-	-	+	+	-	-	+	+	+	-	-	+	+	-	-	+	+	+	-	-	+	+	+	-	-	20
Fibrosarcoma															X													1
Lymph nodes	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	15
Fibrous histiocytoma, metastatic																												1
Thymus	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	6
CIRCULATORY SYSTEM																												
Heart	-	-	+	-	+	+	+	+	+	-	-	+	-	-	-	-	-	-	+	+	+	-	+	+	-			22
DIGESTIVE SYSTEM																												
Oral cavity	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	X	N	N	N	N	N		*50
Squamous cell papilloma																					X							1
Salivary gland	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		6
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		50
Neoplastic nodule												X	X													X		3
Fibrous histiocytoma, metastatic																												1
Skin duct	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		50
Pancreas	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		6
Esophagus	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		6
Stomach	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		7
Squamous cell papilloma																												1
Small intestine	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		7
Large intestine	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		6
Fibrosarcoma									X																			1
URINARY SYSTEM																												
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		50
Mesothelioma, NOS																												1
Urinary bladder	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		6
ENDOCRINE SYSTEM																												
Pituitary	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	+	-	-				12
Adenoma, NOS						X																X						1
Adrenal	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	+	-	-	-	-	-				10
Pheochromocytoma															X				X									4
Pheochromocytoma, malignant																												1
Thyroid	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+				50
C-cell adenoma			X		X									X				X		X	X							7
Parathyroid	+	+	+	+	+	+	+	+	+	+	+	-	+	-	+	+	+	+	+	+	+	+	+	+				43
REPRODUCTIVE SYSTEM																												
Mammary gland	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	+	N	N	N	N	N	N				*50
Fibroadenoma																		X										1
Testis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+				50
Interstitial cell tumor	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X				48
Prostate	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-				6
Preputial/clitoral gland	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N				*50
Carcinoma, NOS																							X					1
NERVOUS SYSTEM																												
Brain	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-				6
BODY CAVITIES																												
Tunica vaginalis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+				*50
Mesothelioma, NOS												X																2
Mesentery	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N				*50
Mesothelioma, NOS												X																2
ALL OTHER SYSTEMS																												
Multiple organs, NOS	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N				*50
Mesothelioma, NOS																												1
Leukemia, mononuclear cell		X	X		X	X			X	X	X			X			X	X	X		X	X	X					20

* Animals necropsied

TABLE A2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF MALE RATS IN THE TWO-YEAR FEED STUDY OF *N*-PHENYL-2-NAPHTHYLAMINE: HIGH DOSE

| ANIMAL NUMBER | 0 0 0

TABLE A2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF MALE RATS: HIGH DOSE (Continued)

ANIMAL NUMBER / WEEKS ON STUDY	TOTAL: TISSUES TUMORS
INTEGUMENTARY SYSTEM	
Skin	*50
Squamous cell carcinoma	1
Subcutaneous tissue	*50
Fibroma	1
RESPIRATORY SYSTEM	
Lungs and bronchi	48
Alveolar/bronchiolar adenoma	4
Trachea	49
HEMATOPOIETIC SYSTEM	
Bone marrow	50
Spleen	49
Sarcoma, NOS	1
Lymph nodes	48
Thymus	39
CIRCULATORY SYSTEM	
Heart	49
DIGESTIVE SYSTEM	
Salivary gland	49
Liver	50
Bile duct	50
Pancreas	50
Acinar cell adenoma	1
Esophagus	49
Stomach	49
Small intestine	49
Large intestine	49
URINARY SYSTEM	
Kidney	50
Tubular cell adenoma	1
Urinary bladder	49
ENDOCRINE SYSTEM	
Pituitary	49
Adenoma, NOS	7
Adrenal	50
Cortical adenoma	1
Pheochromocytoma	23
Thyroid	49
Follicular cell adenoma	1
Follicular cell carcinoma	1
C-cell adenoma	4
Parathyroid	48
Adenoma, NOS	1
REPRODUCTIVE SYSTEM	
Mammary gland	*50
Testis	50
Interstitial cell tumor	47
Prostate	50
Penis	*50
Squamous cell carcinoma	1
Preputial/clitoral gland	*50
Adenoma, NOS	1
NERVOUS SYSTEM	
Brain	50
SPECIAL SENSE ORGANS	
Zymbal gland	*50
Carcinoma, NOS	1
BODY CAVITIES	
Peritoneum	*50
Mesothelioma, metastatic	1
Tunica vaginalis	*50
Mesothelioma, malignant	1
ALL OTHER SYSTEMS	
Multiple organs, NOS	*50
Mesothelioma, metastatic	1
Leukemia, mononuclear cell	24

* Animals necropsied

TABLE A3. ANALYSES OF PRIMARY TUMORS IN MALE RATS IN THE TWO-YEAR FEED STUDY OF *N*-PHENYL-2-NAPHTHYLAMINE

	Control	2,500 ppm	5,000 ppm
Skin: Keratoacanthoma			
Overall Rates (a)	4/50 (8%)	1/50 (2%)	0/50 (0%)
Adjusted Rates (b)	13.9%	2.9%	0.0%
Terminal Rates (c)	3/24 (8%)	0/28 (0%)	0/24 (0%)
Week of First Observation	96	101	
Life Table Tests (d)	P=0.014N	P=0.127N	P=0.036N
Incidental Tumor Tests (d)	P=0.018N	P=0.122N	P=0.048N
Cochran-Armitage Trend Test (d)	P=0.036N		
Fisher Exact Test (d)		P=0.181N	P=0.059N
Lung: Alveolar/Bronchiolar Adenoma			
Overall Rates (a)	1/50 (2%)	(e) 0/0 (0%)	4/49 (8%)
Adjusted Rates (b)	4.2%		11.8%
Terminal Rates (c)	1/24 (4%)		4/24 (12%)
Week of First Observation	104		104
Life Table Test (d)			P=0.206
Incidental Tumor Test (d)			P=0.206
Fisher Exact Test (d)			P=0.175
Lung: Alveolar/Bronchiolar Carcinoma			
Overall Rates (a)	3/50 (6%)	(e) 0/0 (0%)	0/49 (0%)
Adjusted Rates (b)	8.7%		0.0%
Terminal Rates (c)	1/24 (4%)		0/24 (0%)
Week of First Observation	66		
Life Table Test (d)			P=0.093N
Incidental Tumor Test (d)			P=0.221N
Fisher Exact Test (d)			P=0.125N
Lung: Alveolar/Bronchiolar Adenoma or Carcinoma			
Overall Rates (a)	4/50 (8%)	(e) 0/0 (0%)	4/49 (8%)
Adjusted Rates (b)	12.6%		11.8%
Terminal Rates (c)	2/24 (8%)		4/24 (12%)
Week of First Observation	66		104
Life Table Test (d)			P=0.480N
Incidental Tumor Test (d)			P=0.638N
Fisher Exact Test (d)			P=0.631
Hematopoietic System: Mononuclear Cell Leukemia			
Overall Rates (a)	21/50 (42%)	(e,f) 29/50 (58%)	24/50 (48%)
Adjusted Rates (b)	46.5%	69.2%	54.6%
Terminal Rates (c)	10/24 (42%)	16/28 (57%)	16/24 (44%)
Week of First Observation	88	80	72
Life Table Tests (d)	P=0.283N	P=0.316	P=0.350N
Incidental Tumor Tests (d)	P=0.414	P=0.173	P=0.426
Cochran-Armitage Trend Test (d)	P=0.269		
Fisher Exact Test (d)		P=0.081	P=0.544
Liver: Neoplastic Nodule			
Overall Rates (a)	0/50 (0%)	3/50 (6%)	0/50 (0%)
Adjusted Rates (b)	0.0%	10.7%	0.0%
Terminal Rates (c)	0/24 (0%)	3/28 (11%)	0/24 (0%)
Week of First Observation		104	
Life Table Tests (d)	P=0.543N	P=0.148	(g)
Incidental Tumor Tests (d)	P=0.543N	P=0.148	(g)
Cochran-Armitage Trend Test (d)	P=0.568		
Fisher Exact Test (d)		P=0.121	(g)

TABLE A3. ANALYSIS OF PRIMARY TUMORS IN MALE RATS IN THE TWO-YEAR FEED STUDY OF *N*-PHENYL-2-NAPHTHYLAMINE (Continued)

	Control	2,500 ppm	5,000 ppm
Liver: Neoplastic Nodule or Hepatocellular Carcinoma			
Overall Rates (a)	2/50 (4%)	3/50 (6%)	0/50 (0%)
Adjusted Rates (b)	8.3%	10.7%	0.0%
Terminal Rates (c)	2/24 (8%)	3/28 (11%)	0/34 (0%)
Week of First Observation	104	104	
Life Table Tests (d)	P=0.120N	P=0.671	P=0.165N
Incidental Tumor Tests (d)	P=0.120N	P=0.671	P=0.165N
Cochran-Armitage Trend Test (d)	P=0.202N		
Fisher Exact Test (d)		P=0.500	P=0.247N
Kidney: Tubular Cell Adenoma or Adenocarcinoma			
Overall Rates (a)	3/50 (6%)	0/50 (0%)	1/50 (2%)
Adjusted Rates (b)	11.7%	0.0%	2.9%
Terminal Rates (c)	2/24 (8%)	0/28 (0%)	0/34 (0%)
Week of First Observation	103		102
Life Table Tests (d)	P=0.115N	P=0.094N	P=0.300N
Incidental Tumor Tests (d)	P=0.143N	P=0.092N	P=0.361N
Cochran-Armitage Trend Test (d)	P=0.176N		
Fisher Exact Test (d)		P=0.121N	P=0.309N
Pituitary Gland: Adenoma			
Overall Rates (a)	14/49 (29%)	(e) 5/12 (42%)	7/49 (14%)
Adjusted Rates (b)	44.4%		18.8%
Terminal Rates (c)	8/24 (33%)		5/34 (15%)
Week of First Observation	81		93
Life Table Test (d)			P=0.016N
Incidental Tumor Test (d)			P=0.040N
Fisher Exact Test (d)			P=0.009N
Pituitary Gland: Adenoma or Carcinoma			
Overall Rates (a)	15/49 (31%)	(e) 5/12 (42%)	7/49 (14%)
Adjusted Rates (b)	47.8%		18.8%
Terminal Rates (c)	9/24 (38%)		5/34 (15%)
Week of First Observation	81		93
Life Table Test (d)			P=0.009N
Incidental Tumor Test (d)			P=0.022N
Fisher Exact Test (d)			P=0.044N
Adrenal Gland: Pheochromocytoma			
Overall Rates (a)	12/50 (24%)	(e) 4/10 (40%)	20/50 (40%)
Adjusted Rates (b)	41.8%		51.0%
Terminal Rates (c)	8/24 (33%)		15/34 (44%)
Week of First Observation	98		94
Life Table Test (d)			P=0.345
Incidental Tumor Test (d)			P=0.214
Fisher Exact Test (d)			P=0.068
Adrenal Gland: Pheochromocytoma or Malignant Pheochromocytoma			
Overall Rates (a)	12/50 (24%)	(e) 5/10 (50%)	20/50 (40%)
Adjusted Rates (b)	41.8%		51.0%
Terminal Rates (c)	8/24 (33%)		15/34 (44%)
Week of First Observation	98		94
Life Table Test (d)			P=0.345
Incidental Tumor Test (d)			P=0.214
Fisher Exact Test (d)			P=0.068

TABLE A3. ANALYSES OF PRIMARY TUMORS IN MALE RATS IN THE TWO-YEAR FEED STUDY OF *N*-PHENYL-2-NAPHTHYLAMINE (Continued)

	Control	2,500 ppm	5,000 ppm
Thyroid Gland: C-Cell Adenoma			
Overall Rates (a)	7/49 (14%)	7/50 (14%)	4/49 (8%)
Adjusted Rates (b)	23.9%	23.2%	11.8%
Terminal Rates (c)	4/24 (17%)	6/28 (21%)	4/34 (12%)
Week of First Observation	70	90	104
Life Table Tests (d)	P=0.088N	P=0.490N	P=0.120N
Incidental Tumor Tests (d)	P=0.153N	P=0.580N	P=0.213N
Cochran-Armitage Trend Test (d)	P=0.220N		
Fisher Exact Test (d)		P=0.597N	P=0.262N
Thyroid Gland: C-Cell Adenoma or Carcinoma			
Overall Rates (a)	9/49 (18%)	7/50 (14%)	4/49 (8%)
Adjusted Rates (b)	31.2%	23.2%	11.8%
Terminal Rates (c)	6/24 (25%)	6/28 (21%)	4/34 (12%)
Week of First Observation	70	90	104
Life Table Tests (d)	P=0.025N	P=0.269N	P=0.036N
Incidental Tumor Tests (d)	P=0.049N	P=0.242N	P=0.072N
Cochran-Armitage Trend Test (d)	P=0.092N		
Fisher Exact Test (d)		P=0.376N	P=0.116N
Testis: Interstitial Cell Tumor			
Overall Rates (a)	43/50 (86%)	49/50 (98%)	47/49 (96%)
Adjusted Rates (b)	100.0%	100.0%	97.9%
Terminal Rates (c)	24/24 (100%)	28/28 (100%)	33/34 (97%)
Week of First Observation	66	62	72
Life Table Tests (d)	P=0.006N	P=0.484N	P=0.084N
Incidental Tumor Tests (d)	P=0.313	P=0.164	P=0.536
Cochran-Armitage Trend Test (d)	P=0.037		
Fisher Exact Test (d)		P=0.030	P=0.084
All Sites: Mesothelioma			
Overall Rates (a)	3/50 (6%)	3/50 (6%)	2/50 (4%)
Adjusted Rates (b)	10.1%	8.1%	5.6%
Terminal Rates (c)	1/24 (4%)	1/28 (4%)	1/34 (3%)
Week of First Observation	87	70	103
Life Table Tests (d)	P=0.296N	P=0.588N	P=0.370N
Incidental Tumor Tests (d)	P=0.471N	P=0.626	P=0.504N
Cochran-Armitage Trend Test (d)	P=0.412N		
Fisher Exact Test (d)		P=0.661N	P=0.500N
All Sites: Benign Tumors			
Overall Rates (a)	47/50 (94%)	49/50 (98%)	48/50 (96%)
Adjusted Rates (b)	100.0%	100.0%	100.0%
Terminal Rates (c)	24/24 (100%)	28/28 (100%)	34/34 (100%)
Week of First Observation	62	62	72
Life Table Tests (d)	P=0.032N	P=0.247N	P=0.029N
Incidental Tumor Tests (d)	P=0.277N	P=0.731	P=0.327N
Cochran-Armitage Trend Test (d)	P=0.389		
Fisher Exact Test (d)		P=0.309	P=0.500
All Sites: Malignant Tumors			
Overall Rates (a)	36/50 (72%)	34/50 (68%)	29/50 (58%)
Adjusted Rates (b)	78.1%	76.6%	62.4%
Terminal Rates (c)	15/24 (63%)	18/28 (64%)	17/34 (50%)
Week of First Observation	36	62	72
Life Table Tests (d)	P=0.012N	P=0.184N	P=0.018N
Incidental Tumor Tests (d)	P=0.171N	P=0.469N	P=0.248N
Cochran-Armitage Trend Test (d)	P=0.085N		
Fisher Exact Test (d)		P=0.414N	P=0.104N

TABLE A3. ANALYSIS OF PRIMARY TUMORS IN MALE RATS IN THE TWO-YEAR FEED STUDY OF *N*-PHENYL-2-NAPHTHYLAMINE (Continued)

	Control	2,500 ppm	5,000 ppm
All Sites: All Tumors			
Overall Rates (a)	50/50 (100%)	50/50 (100%)	50/50 (100%)
Adjusted Rates (b)	100.0%	100.0%	100.0%
Terminal Rates (c)	24/24 (100%)	28/28 (100%)	34/34 (100%)
Week of First Observation	35	62	72
Life Table Tests (d)	P=0.017N	P=0.167N	P=0.024N
Incidental Tumor Tests (d)	(h)	(h)	(h)
Cochran-Armitage Trend Test (d)	(h)		
Fisher Exact Test (d)		P=1.000	P=1.000

(a) Number of tumor-bearing animals/number of animals examined at the site
(b) Kaplan-Meier estimated tumor incidences at the end of the study after adjusting for intercurrent mortality
(c) Observed tumor incidence at terminal kill
(d) Beneath the control incidence are the P values associated with the trend test. Beneath the dosed group incidence are the P values corresponding to pairwise comparisons between that dosed group and the controls. The life table analysis regards tumors in animals dying prior to terminal kill as being (directly or indirectly) the cause of death. The incidental tumor test regards these lesions as nonfatal. The Cochran-Armitage and Fisher exact tests compare directly the overall incidence rates. A negative trend or lower incidence in a dosed group is indicated by (N).
(e) Incomplete sampling of tissues
(f) Only 30 spleens, 15 lymph nodes, 6 thymuses, and 7 small intestines were examined.
(g) No P value is reported because no tumors were observed in the 5,000-ppm and control groups.
(h) No P value is reported because all animals had tumors.

TABLE A4a. HISTORICAL INCIDENCE OF SPLENIC TUMORS IN MALE F344/N RATS RECEIVING NO TREATMENT (a)

	Incidence of Sarcomas in Controls
Historical Incidence at Battelle Columbus Laboratories	
	0/236
Overall Historical Incidence	
TOTAL	(b) 5/1,954 (0.3%)
SD (c)	0.70%
Range (d)	
High	1/45
Low	0/90

(a) Data as of August 30, 1985, for studies of at least 104 weeks
(b) No fibrosarcomas have been observed.
(c) Standard deviation
(d) Range and SD are presented for groups of 35 or more animals.

TABLE A4b. HISTORICAL INCIDENCE OF LARGE INTESTINE TUMORS IN MALE F344/N RATS RECEIVING NO TREATMENT (a)

	Incidence of Fibromas in Controls
Historical Incidence at Battelle Columbus Laboratories	
	0/227
Overall Historical Incidence	
TOTAL	(b) 1/1,879 (<0.1%)
SD (c)	0.32%
Range (d)	
High	1/50
Low	0/87

(a) Data as of August 30, 1985, for studies of at least 104 weeks
(b) No sarcomas or fibrosarcomas have been observed.
(c) Standard deviation
(d) Range and SD are presented for groups of 35 or more animals.

TABLE A4c. HISTORICAL INCIDENCE OF THYROID GLAND C-CELL TUMORS IN MALE F344/N RATS RECEIVING NO TREATMENT (a)

Study	Incidence in Controls		
	Adenoma	Carcinoma	Adenoma or Carcinoma
Historical Incidence at Battelle Columbus Laboratories			
Chlorobenzene	0/49	6/49	6/49
Common control group (b)	0/89	2/89	2/89
C.I. Disperse Yellow 3	0/49	4/49	4/49
D&C Red No. 9	3/50	2/50	5/50
C.I. Solvent Yellow 14	0/50	3/50	3/50
l-Ascorbic acid	2/49	4/49	5/49
TOTAL	5/336 (1.5%)	21/336 (6.3%)	25/336 (7.4%)
SD (c)	2.67%	3.54%	3.57%
Range (d)			
High	3/50	6/49	6/49
Low	0/89	2/89	2/89
Overall Historical Incidence			
TOTAL	122/1,928 (6.3%)	72/1,928 (3.7%)	192/1,928 (10.0%)
SD (c)	5.22%	3.57%	6.04%
Range (d)			
High	10/50	6/49	15/50
Low	0/89	0/50	1/50

(a) Data as of August 30, 1985, for studies of at least 104 weeks
(b) Ninety-animal common control group for the C.I. Acid Orange 10, FD&C Yellow No. 6, and C.I. Acid Red 14 studies
(c) Standard deviation
(d) Range and SD are presented for groups of 35 or more animals.

TABLE A5. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE RATS IN THE TWO-YEAR FEED STUDY OF *N*-PHENYL-2-NAPHTHYLAMINE

	Untreated Control	Low Dose	High Dose
ANIMALS INITIALLY IN STUDY	50	50	50
ANIMALS NECROPSIED	50	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY	50	50	50
INTEGUMENTARY SYSTEM			
*Skin	(50)	(50)	(50)
Epidermal inclusion cyst		2 (4%)	1 (2%)
Hyperkeratosis	2 (4%)		
Acanthosis	1 (2%)		
*Subcutaneous tissue	(50)	(50)	(50)
Edema, NOS			1 (2%)
Inflammation, acute/chronic		1 (2%)	
RESPIRATORY SYSTEM			
*Nasal mucosa	(50)	(50)	(50)
Inflammation, acute		1 (2%)	
*Nasal turbinate	(50)	(50)	(50)
Inflammation, acute/chronic	16 (32%)		14 (28%)
Hyperplasia, epithelial	2 (4%)		
#Trachea	(50)	(6)	(49)
Inflammation, chronic	1 (2%)		
#Lung	(50)	(9)	(49)
Lymphocytic inflammatory infiltration	1 (2%)		
Inflammation, acute diffuse			1 (2%)
Inflammation, acute/chronic		1 (11%)	1 (2%)
Inflammation, chronic	2 (4%)		
Hyperplasia, epithelial	1 (2%)		5 (10%)
HEMATOPOIETIC SYSTEM			
#Bone marrow	(49)	(6)	(50)
Myelofibrosis	1 (2%)	2 (33%)	1 (2%)
#Spleen	(49)	(30)	(49)
Infarct, acute		1 (3%)	
#Splenic capsule	(49)	(30)	(49)
Fibrosis	1 (2%)		
#Splenic red pulp	(49)	(30)	(49)
Fibrosis	4 (8%)	5 (17%)	5 (10%)
Hyperplasia, reticulum cell	1 (2%)		
Hematopoiesis	2 (4%)		
#Mandibular lymph node	(47)	(15)	(48)
Plasmacytosis	1 (2%)		
#Cervical lymph node	(47)	(15)	(48)
Plasmacytosis			1 (2%)
#Mesenteric lymph node	(47)	(15)	(48)
Hemorrhage		1 (7%)	
Inflammation, chronic		1 (7%)	
#Renal lymph node	(47)	(15)	(48)
Pigmentation, NOS		1 (7%)	
#Lung	(50)	(9)	(49)
Leukocytosis, NOS		1 (11%)	
#Liver	(50)	(50)	(50)
Hematopoiesis	1 (2%)		
#Thymus	(35)	(6)	(39)
Depletion, lymphoid	25 (71%)		37 (95%)

TABLE A6. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE RATS IN THE TWO-YEAR FEED STUDY OF *N*-PHENYL-2-NAPHTHYLAMINE (Continued)

	Untreated Control	Low Dose	High Dose
CIRCULATORY SYSTEM			
#Splenic red pulp	(49)	(50)	(48)
Thrombus, organized	1 (2%)		
#Heart/atrium	(50)	(22)	(49)
Dilatation, NOS		1 (5%)	
Thrombosis, NOS	1 (2%)	4 (18%)	4 (8%)
Inflammation, chronic focal			1 (2%)
#Myocardium	(50)	(22)	(49)
Degeneration, NOS	48 (96%)	17 (77%)	48 (98%)
*Artery	(50)	(50)	(50)
Mineralization		1 (2%)	
*Pulmonary artery	(50)	(50)	(50)
Thrombus, organized	1 (2%)		
*Splenic artery	(50)	(50)	(50)
Thrombosis, NOS	1 (2%)		
*Sup. pancreaticoduodenal artery	(50)	(50)	(50)
Inflammation, acute/chronic			3 (6%)
Inflammation, chronic	1 (2%)		3 (6%)
#Periesophageal tissue	(50)	(6)	(48)
Perivasculitis	1 (2%)		
DIGESTIVE SYSTEM			
*Periodontal tissues	(50)	(50)	(50)
Inflammation, acute/chronic			1 (2%)
Hyperkeratosis			1 (2%)
Acanthosis			1 (2%)
#Salivary gland	(48)	(6)	(48)
Necrosis, diffuse			1 (2%)
#Liver	(50)	(50)	(50)
Hernia, NOS		1 (2%)	
Congestion, NOS	1 (2%)	1 (2%)	
Inflammation, acute/chronic	4 (8%)	1 (2%)	2 (4%)
Degeneration, cystic	15 (30%)	26 (52%)	15 (30%)
Necrosis, NOS	2 (4%)	4 (8%)	2 (4%)
Basophilic cyto change	22 (44%)	26 (52%)	38 (76%)
Focal cellular change	1 (2%)		
Eosinophilic cyto change	3 (6%)	1 (2%)	
Clear cell change	3 (6%)	1 (2%)	2 (4%)
Hyperplasia, focal		1 (2%)	
#Liver/centrilobular	(50)	(50)	(50)
Degeneration, NOS	1 (2%)		
Necrosis, NOS	1 (2%)	3 (6%)	1 (2%)
Cytoplasmic vacuolization	2 (4%)	1 (2%)	13 (26%)
#Bile duct	(50)	(50)	(50)
Hyperplasia, NOS	40 (80%)	32 (64%)	37 (74%)
#Pancreas	(46)	(9)	(50)
DilatationAducts	1 (2%)	1 (11%)	
#Pancreatic acinus	(46)	(9)	(50)
Inflammation, chronic diffuse			1 (2%)
Fibrosis, focal			1 (2%)
Atrophy, NOS	11 (24%)	6 (50%)	12 (24%)
#Glandular stomach	(49)	(7)	(49)
Mineralization	1 (2%)	1 (14%)	
Edema, NOS	1 (2%)		
Ulcer, acute			2 (4%)
Inflammation, acute/chronic	1 (2%)		
Necrosis, NOS	1 (2%)		1 (2%)

TABLE A5. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE RATS IN THE TWO-YEAR FEED STUDY OF *N*-PHENYL-2-NAPHTHYLAMINE (Continued)

	Untreated Control	Low Dose	High Dose
DIGESTIVE SYSTEM (Continued)			
#Gastric submucosa	(49)	(7)	(49)
Edema, NOS			1 (2%)
Inflammation, acute focal			1 (2%)
Inflammation, acute/chronic	1 (2%)		1 (2%)
#Forestomach	(49)	(7)	(49)
Ulcer, acute	1 (2%)		1 (2%)
Inflammation, acute/chronic	2 (4%)	1 (14%)	
Hyperkeratosis	1 (2%)		
Acanthosis	1 (2%)		
#Pylorus	(49)	(7)	(49)
Hyperplasia, epithelial	1 (2%)		
#Duodenum	(48)	(7)	(46)
Ulcer, acute			1 (2%)
Inflammation, acute focal	1 (2%)		
Necrosis, focal	2 (4%)		
#Jejunum	(48)	(7)	(46)
Congestion, NOS		1 (14%)	
#Colon	(48)	(8)	(48)
Parasitism	3 (6%)		2 (4%)
*Rectum	(50)	(50)	(50)
Inflammation, chronic			1 (2%)
Parasitism	4 (8%)		
URINARY SYSTEM			
#Kidney	(50)	(50)	(50)
Mineralization	1 (2%)	2 (4%)	2 (4%)
Hydronephrosis	2 (4%)		
Cyst, NOS		5 (10%)	5 (10%)
Nephropathy	50 (100%)	50 (100%)	50 (100%)
Hyperplasia, tubular cell		1 (2%)	
#Kidney/tubule	(50)	(50)	(50)
Inflammation, acute suppurative	8 (16%)	32 (64%)	40 (80%)
Pigmentation, NOS		1 (2%)	
#Kidney/pelvis	(50)	(50)	(50)
Hyperplasia, epithelial	1 (2%)	2 (4%)	1 (2%)
*Ureter	(50)	(50)	(50)
Inflammation, acute/chronic	1 (2%)		
#Urinary bladder	(48)	(6)	(49)
Dilatation, NOS			1 (2%)
Inflammation, acute focal			1 (2%)
Inflammation, acute necrotizing			1 (2%)
Inflammation, acute/chronic	1 (2%)		
#Urinary bladder/mucosa	(48)	(6)	(49)
Hyperplasia, epithelial	1 (2%)		
ENDOCRINE SYSTEM			
#Pituitary intermedia	(49)	(12)	(49)
Cyst, NOS	1 (2%)		
Multiple cysts			1 (2%)
Hyperplasia, focal	1 (2%)		
#Anterior pituitary	(49)	(12)	(49)
Cyst, NOS	4 (8%)	2 (17%)	5 (10%)
Multiple cysts	3 (6%)		5 (10%)
Hemorrhage, chronic	1 (2%)		
Inflammation, chronic focal			1 (2%)
Cytoplasmic vacuolization			1 (2%)
Hyperplasia, NOS	9 (18%)	2 (17%)	10 (20%)
Angiectasis			2 (4%)

TABLE A5. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE RATS IN THE TWO-YEAR FEED STUDY OF *N*-PHENYL-2-NAPHTHYLAMINE (Continued)

	Untreated Control	Low Dose	High Dose
ENDOCRINE SYSTEM (Continued)			
#Adrenal/capsule	(50)	(10)	(50)
Ectopia	1 (2%)		
Hyperplasia, NOS	1 (2%)		
#Adrenal cortex	(50)	(10)	(50)
Degeneration, NOS	3 (6%)		1 (2%)
Necrosis, NOS			1 (2%)
Necrosis, focal			1 (2%)
Metamorphosis, fatty	2 (4%)	1 (10%)	2 (4%)
Hypertrophy, NOS	1 (2%)		1 (2%)
Hyperplasia, NOS	15 (30%)	1 (10%)	23 (46%)
#Adrenal medulla	(50)	(10)	(50)
Hyperplasia, NOS	15 (30%)	1 (10%)	11 (22%)
#Thyroid	(49)	(50)	(49)
Follicular cyst, NOS		1 (2%)	1 (2%)
Multiple cysts			1 (2%)
Hyperplasia, C-cell	38 (78%)	39 (78%)	43 (88%)
Hyperplasia, follicular cell			1 (2%)
#Parathyroid	(39)	(43)	(43)
Hyperplasia, NOS	2 (5%)	4 (9%)	9 (21%)
REPRODUCTIVE SYSTEM			
*Mammary gland	(50)	(50)	(50)
Hyperplasia, cystic	30 (60%)		32 (64%)
*Preputial gland	(50)	(50)	(50)
Dilatation/ducts			3 (6%)
Inflammation, suppurative			1 (2%)
Inflammation, acute/chronic	37 (74%)	1 (2%)	41 (82%)
Hyperplasia, focal			2 (4%)
#Prostate	(49)	(6)	(49)
Inflammation, acute/chronic	10 (20%)	1 (17%)	11 (22%)
*Seminal vesicle	(50)	(50)	(50)
Inflammation, acute/chronic			1 (2%)
#Testis	(50)	(50)	(49)
Atrophy, NOS	42 (84%)	48 (96%)	43 (88%)
Hyperplasia, interstitial cell	12 (24%)	8 (16%)	17 (35%)
*Epididymis	(50)	(50)	(50)
Inflammation, acute/chronic	2 (4%)		1 (2%)
NERVOUS SYSTEM			
#Cerebral ventricle	(50)	(6)	(50)
Hydrocephalus, NOS	4 (8%)		
#Brain	(50)	(6)	(50)
Hemorrhage	3 (6%)		
Inflammation, acute/chronic	1 (2%)		
Necrosis, NOS	1 (2%)		
Atrophy, pressure	2 (4%)		1 (2%)
SPECIAL SENSE ORGANS			
*Eye/retina	(50)	(50)	(50)
Atrophy, NOS	2 (4%)	1 (2%)	2 (4%)
*Eye/crystalline lens	(50)	(50)	(50)
Cataract	2 (4%)	1 (2%)	1 (2%)
*Nasolacrimal duct	(50)	(50)	(50)
Inflammation, acute/chronic			2 (4%)
Hyperkeratosis			1 (2%)
*Harderian gland	(50)	(50)	(50)
Hyperplasia, epithelial	1 (2%)		

TABLE A5. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE RATS IN THE TWO-YEAR FEED STUDY OF *N*-PHENYL-2-NAPHTHYLAMINE (Continued)

	Untreated Control	Low Dose	High Dose
SPECIAL SENSE ORGANS (Continued)			
*Middle ear	(50)	(50)	(50)
Inflammation, acute/chronic	1 (2%)		
*Internal ear	(50)	(50)	(50)
Inflammation, acute/chronic	1 (2%)		
MUSCULOSKELETAL SYSTEM			
*Maxilla	(50)	(50)	(50)
Inflammation, acute/chronic	1 (2%)		
BODY CAVITIES			
*Mediastinum	(50)	(50)	(50)
Inflammation, acute/chronic			1 (2%)
*Visceral peritoneum	(50)	(50)	(50)
Inflammation, pyogranulomatous	1 (2%)		
*Mesentery	(50)	(50)	(50)
Inflammation, acute/chronic		2 (4%)	
Inflammation, chronic	3 (6%)		1 (2%)
ALL OTHER SYSTEMS			
*Multiple organs	(50)	(50)	(50)
Mineralization	1 (2%)		
SPECIAL MORPHOLOGY SUMMARY			
None			

* Number of animals receiving complete necropsy examinations; all gross lesions including masses examined microscopically.
# Number of animals examined microscopically at this site

APPENDIX B

SUMMARY OF LESIONS IN FEMALE RATS IN THE TWO-YEAR FEED STUDY OF *N*-PHENYL-2-NAPHTHYLAMINE

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TABLE B1. SUMMARY OF THE INCIDENCE OF NEOPLASMS IN FEMALE RATS IN THE TWO-YEAR FEED STUDY OF *N*-PHENYL-2-NAPHTHYLAMINE

	Untreated Control	Low Dose	High Dose
ANIMALS INITIALLY IN STUDY	50	50	50
ANIMALS NECROPSIED	50	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY	50	50	50
INTEGUMENTARY SYSTEM			
*Multiple organs	(50)	(50)	(50)
Fibrous histiocytoma, malignant			1 (2%)
*Skin	(50)	(50)	(50)
Trichoepithelioma	1 (2%)		
*Subcutaneous tissue	(50)	(50)	(50)
Sarcoma, NOS			1 (2%)
Fibroma	1 (2%)		
Fibrosarcoma	1 (2%)		
RESPIRATORY SYSTEM			
#Lung	(50)	(4)	(50)
Alveolar/bronchiolar adenoma			3 (6%)
HEMATOPOIETIC SYSTEM			
*Multiple organs	(50)	(50)	(50)
Leukemia, mononuclear cell	14 (28%)	6 (12%)	6 (12%)
CIRCULATORY SYSTEM			
#Spleen	(49)	(3)	(50)
Hemangiosarcoma	1 (2%)		
DIGESTIVE SYSTEM			
#Liver	(50)	(50)	(50)
Neoplastic nodule	1 (2%)		
Hepatocellular carcinoma	1 (2%)		
#Jejunum	(49)	(5)	(49)
Adenomatous polyp, NOS		1 (20%)	
#Colon	(49)	(4)	(48)
Fibrosarcoma		1 (25%)	
URINARY SYSTEM			
None			
ENDOCRINE SYSTEM			
#Pituitary intermedia	(50)	(25)	(49)
Adenoma, NOS	1 (2%)	1 (4%)	
#Pituitary pars intermedia	(50)	(25)	(49)
Adenoma, NOS		1 (4%)	
#Anterior pituitary	(50)	(25)	(49)
Carcinoma, NOS	1 (2%)		
Adenoma, NOS	31 (62%)	16 (64%)	14 (29%)
#Adrenal	(50)	(7)	(50)
Cortical adenoma	1 (2%)		
#Adrenal medulla	(50)	(7)	(50)
Pheochromocytoma	4 (8%)	2 (29%)	1 (2%)
#Thyroid	(50)	(49)	(50)
Follicular cell adenoma		1 (2%)	
Follicular cell carcinoma			1 (2%)
C-cell adenoma	17 (34%)	11 (22%)	1 (2%)
C-cell carcinoma	3 (6%)		

TABLE B1. SUMMARY OF THE INCIDENCE OF NEOPLASMS IN FEMALE RATS IN THE TWO-YEAR FEED STUDY OF *N*-PHENYL-2-NAPHTHYLAMINE (Continued)

	Untreated Control	Low Dose	High Dose
ENDOCRINE SYSTEM (Continued)			
#Parathyroid	(38)	(44)	(41)
Adenoma, NOS		1 (2%)	
#Pancreatic islets	(50)	(4)	(50)
Islet cell adenoma	1 (2%)	1 (25%)	
REPRODUCTIVE SYSTEM			
*Mammary gland	(50)	(50)	(50)
Adenocarcinoma, NOS	2 (4%)		
Fibroadenoma	16 (32%)	5 (10%)	5 (10%)
*Clitoral gland	(50)	(50)	(50)
Adenoma, NOS	2 (4%)	1 (2%)	2 (4%)
#Uterus	(50)	(17)	(50)
Endometrial stromal polyp	6 (12%)	7 (41%)	7 (14%)
Neurilemoma, malignant		1 (6%)	
NERVOUS SYSTEM			
None			
SPECIAL SENSE ORGANS			
*Eye/anterior chamber	(50)	(50)	(50)
Sarcoma, NOS			1 (2%)
MUSCULOSKELETAL SYSTEM			
None			
BODY CAVITIES			
None			
ALL OTHER SYSTEMS			
Knee			
Osteoma		1	
ANIMAL DISPOSITION SUMMARY			
Animals initially in study	50	50	50
Natural death	3	4	3
Moribund sacrifice	11	2	9
Terminal sacrifice	36	44	38
TUMOR SUMMARY			
Total animals with primary tumors**	46	36	33
Total primary tumors	105	57	43
Total animals with benign tumors	42	35	26
Total benign tumors	81	49	33
Total animals with malignant tumors	22	8	10
Total malignant tumors	23	8	10
Total animals with tumors uncertain--benign or malignant	1		
Total uncertain tumors	1		

* Number of animals receiving complete necropsy examinations; all gross lesions including masses examined microscopically.
** Primary tumors: all tumors except secondary tumors
# Number of animals examined microscopically at this site

TABLE B3. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF FEMALE RATS IN THE TWO-YEAR FEED STUDY OF *N*-PHENYL-2-NAPHTHYLAMINE: UNTREATED CONTROL

ANIMAL NUMBER	0 0 1 1	0 0 2 0	0 0 2 1	0 0 2 2	0 0 2 3	0 0 2 4	0 0 3 0	0 0 3 1	0 0 3 2	0 0 3 3	0 0 3 4	0 0 3 5	0 0 3 6	0 0 3 7	0 0 3 8	0 0 3 9	0 0 4 0	0 0 4 1	0 0 4 2	0 0 4 3	0 0 4 4	0 0 4 5	0 0 4 6	0 0 4 7	0 0 4 8	0 0 4 9	0 0 5 0
WEEKS ON STUDY	0 0 3	0 0 3	0 1 1	0 2 4	0 2 4	0 2 5	0 3 7	0 4 4	0 4 8	0 5 0	0 5 0	0 5 2	0 5 2	0 5 2	0 5 2	0 5 2	0 5 2	0 5 2	0 5 2	0 5 2	0 5 2	0 5 2	0 5 2	0 5 2	0 5 2	0 5 2	0 5 4
INTEGUMENTARY SYSTEM																											
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trichoepithelioma																											
Subcutaneous tissue	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Fibroma																											
Fibrosarcoma																											
RESPIRATORY SYSTEM																											
Lungs and bronchi	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
HEMATOPOIETIC SYSTEM																											
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spleen	+	+	+	+	+	+	+	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hemangiosarcoma							X																				
Lymph nodes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	+	+	+	+	+	+	+	+	+	+
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
CIRCULATORY SYSTEM																											
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
DIGESTIVE SYSTEM																											
Salivary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Neoplastic nodule																											
Hepatocellular carcinoma																											
Bile duct	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Small intestine	+	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Large intestine	+	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
URINARY SYSTEM																											
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
ENDOCRINE SYSTEM																											
Pituitary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	+	+	+
Carcinoma, NOS																											
Adenoma, NOS			X		X	X		X	X	X	X	X	X			X	X	X			X	X	X			X	X
Adrenal	+	+	+	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	+	+
Cortical adenoma							X																				
Pheochromocytoma																										X	
Thyroid	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	+	+
C-cell adenoma							X						X			X		X			X		X				X
C-cell carcinoma																											
Parathyroid	+	+	–	+	–	+	+	+	+	+	+	+	+	+	+	–	+	+	+	+	+	–	–	+	+	+	+
Pancreatic islets	+	+	+	+	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Islet cell adenoma																	X										
REPRODUCTIVE SYSTEM																											
Mammary gland	N	+	+	+	+	+	+	+	+	+	+	+	+	N	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenocarcinoma, NOS													X		X												
Fibroadenoma				X	X				X	X																X	
Preputial/clitoral gland	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Adenoma, NOS																											
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Endometrial stromal polyp							X						X		X												X
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
NERVOUS SYSTEM																											
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
ALL OTHER SYSTEMS																											
Multiple organs, NOS	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Leukemia, mononuclear cell	X						X	X	X		X		X		X	X				X		X	X				

TABLE B2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF FEMALE RATS: UNTREATED CONTROL (Continued)

ANIMAL NUMBER	0 1 1	0 1 2	0 1 3	0 1 4	0 2 1	0 2 2	0 2 3	0 2 4	0 3 1	0 3 2	0 3 3	0 3 4	0 4 1	0 4 2	0 4 3	0 4 4	0 5 1	0 5 2	0 5 3	0 5 4	0 6 1	0 6 2	0 6 3	0 6 4	0 7 1	0 7 2	0 7 3	0 7 4	0 8 1	0 8 2	0 8 3	0 8 4	0 9 1	0 9 2	0 9 3	0 9 4	0 10 1	0 10 2	0 10 3	0 10 4	TOTAL: TISSUES TUMORS
WEEKS ON STUDY	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	
INTEGUMENTARY SYSTEM																																								
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	*50
Trichoepithelioma							X																																	1
Subcutaneous tissue	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	*50
Fibroma																																								1
Fibrosarcoma				X																																				1
RESPIRATORY SYSTEM																																								
Lungs and bronchi	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
HEMATOPOIETIC SYSTEM																																								
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Hemangiosarcoma																																								1
Lymph nodes	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
CIRCULATORY SYSTEM																																								
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
DIGESTIVE SYSTEM																																								
Salivary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Neoplastic nodule			X																																					1
Hepatocellular carcinoma											X																													1
Bile duct	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Small intestine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Large intestine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
URINARY SYSTEM																																								
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
ENDOCRINE SYSTEM																																								
Pituitary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Carcinoma, NOS																																								1
Adenoma, NOS				X	X	X	X	X	X			X		X	X	X	X	X	X		X		X		X															32
Adrenal	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Cortical adenoma																																								1
Pheochromocytoma		X									X					X																									4
Thyroid	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
C-cell adenoma	X			X	X		X						X						X	X			X	X			X			X										17
C-cell carcinoma																			X						X			X													3
Parathyroid	-	-	+	-	+	-	+	+	+	-	+	+	+	+	+	+	+	+	+	+	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	38
Pancreatic islets	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Islet cell adenoma																																								1
REPRODUCTIVE SYSTEM																																								
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	*50
Adenocarcinoma, NOS																																								2
Fibroadenoma	X		X	X			X				X					X					X				X			X			X			X			X			16
Preputial/clitoral gland	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	*50
Adenoma, NOS						X																																		2
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Endometrial stromal polyp				X							X																													6
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
NERVOUS SYSTEM																																								
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
ALL OTHER SYSTEMS																																								
Multiple organs, NOS	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	*50
Leukemia, mononuclear cell			X		X			X								X																								14

* Animals necropsied

TABLE B8. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF FEMALE RATS IN THE TWO-YEAR FEED STUDY OF *N*-PHENYL-2-NAPHTHYLAMINE: LOW DOSE

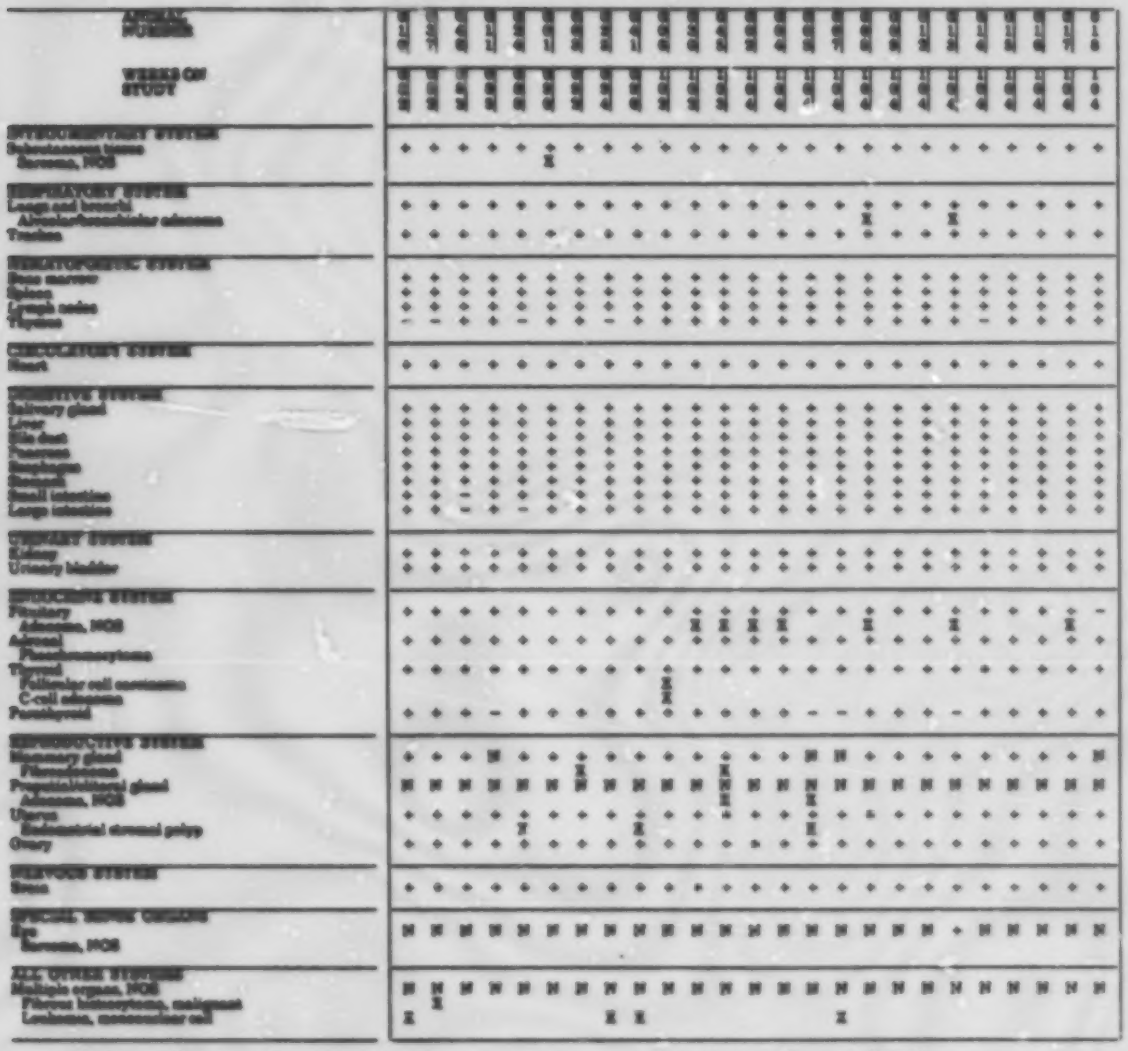
ANIMAL NUMBER	0 4 0	0 7 3	0 8 3	0 8 6	0 9 1	0 9 5	0 9 6	0 9 7	0 9 8	0 9 9	1 0 0	1 0 1	1 0 2	1 0 3	1 0 4	1 0 5	1 0 6	1 0 7	1 0 8	1 0 9	1 1 0	1 1 1	1 1 2	1 1 3	1 1 4	1 1 5	1 1 6	1 1 7	1 1 8	1 1 9	1 2 0
WEEKS ON STUDY	0 3	0 3	0 3	0 3	0 4	0 4	0 4	0 4	0 4	0 4	0 4	0 4	0 4	0 4	0 4	0 4	0 4	0 4	0 4	0 4	0 4	0 4	0 4	0 4	0 4	0 4	0 4	0 4	0 4	0 4	1 0 4
RESPIRATORY SYSTEM																															
Lungs and bronchi	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Trachea	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
HEMATOPOIETIC SYSTEM																															
Bone marrow	-	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Spleen	-	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Lymph nodes	-	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Thymus	-	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
CIRCULATORY SYSTEM																															
Heart	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
DIGESTIVE SYSTEM																															
Salivary gland	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Bile duct	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pancreas	-	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Esophagus	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Stomach	-	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Small intestine	-	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Adenomatous polyp, NOS													X																		
Large intestine	-	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Fibrosarcoma																															X
URINARY SYSTEM																															
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Urinary bladder	-	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
ENDOCRINE SYSTEM																															
Pituitary	-	+	+	+	-	+	+	-	-	-	-	-	+	+	-	+	+	-	+	+	+	-	-	-	-	+	+	-	-	-	-
Adenoma, NOS		X			X	X							X			X	X		X	X	X					X				
Adrenal	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Pheochromocytoma																															
Thyroid	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Follicular cell adenoma													X																		
C-cell adenoma										X	X						X											X			
Parathyroid	+	-	+	+	-	+	-	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+
Adenoma, NOS																															
Pancreatic islets	-	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-
Islet cell adenoma																												X			
REPRODUCTIVE SYSTEM																															
Mammary gland	N	+	+	+	N	N	N	+	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Fibroadenoma			X					X																							
Preputial/clitoral gland	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Adenoma, NOS								X																							
Uterus	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-
Endometrial stromal polyp	X				X											X															
Hemangioma, malignant																															
Ovary	+	+	+	+	-	+	+	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
NERVOUS SYSTEM																															
Brain	-	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
ALL OTHER SYSTEMS																															
Multiple organs, NOS	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Lymphoma, mononuclear cell				X		X														X											
Sarc, NOS																															
Osteoma																															

TABLE B2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF FEMALE RATS: LOW DOSE (Continued)

ANIMAL NUMBER																									TOTAL: TISSUE TUMORS
WEEKS ON STUDY																									
RESPIRATORY SYSTEM																									
Lungs and bronchi	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	4
Trachea																									4
HEMATOPOIETIC SYSTEM																									
Bone marrow	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2
Spleen	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3
Lymph nodes	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3
Thymus	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3
CIRCULATORY SYSTEM																									
Heart	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	4
DIGESTIVE SYSTEM																									
Salivary gland	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	4
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Skin duct	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pancreas	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	4
Esophagus	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	4
Stomach	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3
Small intestine	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3
Adenomatous polyp, NOS																									1
Large intestine	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	4
Fibrosarcoma																									1
URINARY SYSTEM																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Urinary bladder	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3
ENDOCRINE SYSTEM																									
Pituitary	+	-	+	-	+	+	+	-	+	+	-	+	+	+	-	-	-	-	+	-	-	-	-	+	38
Adenoma, NOS			X			X	X		X	X			X	X					X						12
Adrenal	+	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	4
Pheochromocytoma	X																X								2
Thyroid	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
Follicular cell adenoma																									1
C-cell adenoma					X			X	X		X								X			X	X		11
Parathyroid	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	44
Adenoma, NOS																	X								1
Pancreatic islets	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	4
Islet cell adenoma																									1
REPRODUCTIVE SYSTEM																									
Mammary gland	M	M	M	M	M	M	+	M	M	M	M	M	M	M	M	M	M	+	M	+	M	M	M	M	*50
Fibroadenoma							X											X		X					3
Preputial/clitoral gland	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	*50
Adenoma, NOS																							X		1
Uterus	+	+	-	+	-	-	-	-	-	+	-	+	-	-	-	-	+	-	-	+	-	-	-	+	17
Endometrial stromal polyp	X																	X		X				X	7
Hemangioma, malignant				X																					1
Ovary	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	6
NERVOUS SYSTEM																									
Brain	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3
ALL OTHER SYSTEMS																									
Multiple organs, NOS	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	*50
Leukemia, mononuclear cell								X	X										X						8
Knee, NOS																									
Osteoma																	X								1

* Animals necropsied

TABLE B2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF FEMALE RATS IN THE TWO-YEAR FEED STUDY OF *N*-PHENYL-2-NAPHTHYLAMINE: HIGH DOSE



+: Tissue examined microscopically
R: Required tissue not examined microscopically
X: Tumor incidence
B: Necropsy, no autolysis, no microscopic examination
A: Animal missing
C: No tissue information submitted
N: Necropsy, no histology due to protocol
P: Autolysis
M: Animal missing
S: No necropsy performed

TABLE B2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF FEMALE RATS: HIGH DOSE
(Continued)

ANIMAL NUMBER	0 1 2	0 1 3	0 1 4	0 1 5	0 2 1	0 2 2	0 2 3	0 2 4	0 2 5	0 3 1	0 3 2	0 3 3	0 3 4	0 3 5	0 4 1	0 4 2	0 4 3	0 4 4	0 4 5	0 5 1	0 5 2	0 5 3	0 5 4	0 5 5	TOTAL: TISSUES TUMORS
WEEKS ON STUDY	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	
INTEGUMENTARY SYSTEM																								
Subcutaneous tissue	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	*50
Sarcoma, NOS																								1
RESPIRATORY SYSTEM																								
Lungs and bronchi	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar adenoma																	X							1
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
HEMATOPOIETIC SYSTEM																								
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	48
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymph nodes	+	+	+	+	+	+	+	+	–	+	+	+	+	+	+	+	+	+	+	+	+	–	+	48
Thymus	+	+	+	+	–	+	+	+	+	+	+	+	+	+	+	+	–	+	+	+	–	+	+	43
CIRCULATORY SYSTEM																								
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
DIGESTIVE SYSTEM																								
Salivary gland	+	+	+	+	+	+	+	+	–	+	+	+	+	+	+	+	+	+	+	+	+	–	+	48
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Bile duct	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Small intestine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Large intestine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
URINARY SYSTEM																								
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
ENDOCRINE SYSTEM																								
Pituitary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Adenoma, NOS		X		X		X		X												X	X	X		14
Adrenal	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pheochromocytoma															X									1
Thyroid	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Follicular cell carcinoma																								1
C-cell adenoma																								1
Parathyroid	+	+	+	+	–	+	+	+	+	+	+	–	–	+	+	+	+	+	+	+	–	+	–	41
REPRODUCTIVE SYSTEM																								
Mammary gland	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	*50
Fibroadenoma									X											X			X	5
Preputial/clitoral gland	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	*50
Adenoma, NOS																								2
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Endometrial stromal polyp	X						X								X	X								7
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
NERVOUS SYSTEM																								
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
SPECIAL SENSE ORGANS																								
Eye	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	+	M	M	M	M	M	M	*50
Sarcoma, NOS																	X							1
ALL OTHER SYSTEMS																								
Multiple organs, NOS	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	*50
Fibrous histiocytoma, malignant													X	X										1
Leukemia, mononuclear cell																								8

* Animals necropsied

TABLE B3. ANALYSIS OF PRIMARY TUMORS IN FEMALE RATS IN THE TWO-YEAR FEED STUDY OF *N*-PHENYL-2-NAPHTHYLAMINE

	Control	2,500 ppm	5,000 ppm
Lung: Alveolar/Bronchiolar Adenoma			
Overall Rates (a)	0/50 (0%)	(b) 0/4 (0%)	3/50 (6%)
Adjusted Rates (c)	0.0%		7.9%
Terminal Rates (d)	0/36 (0%)		3/38 (8%)
Week of First Observation			104
Life Table Test (e)			P=0.131
Incidental Tumor Test (e)			P=0.131
Fisher Exact Test (e)			P=0.121
Hematopoietic System: Mononuclear Cell Leukemia			
Overall Rates (a)	14/50 (28%)	(b,f) 6/50 (12%)	6/50 (12%)
Adjusted Rates (c)	32.0%		13.9%
Terminal Rates (d)	7/36 (19%)		3/38 (8%)
Week of First Observation	38		82
Life Table Test (e)			P=0.044N
Incidental Tumor Test (e)			P=0.062N
Fisher Exact Test (e)			P=0.039N
Pituitary Gland: Adenoma			
Overall Rates (a)	31/50 (62%)	(b) 16/25 (64%)	14/49 (29%)
Adjusted Rates (c)	68.7%		35.8%
Terminal Rates (d)	22/36 (61%)		12/37 (32%)
Week of First Observation	61		102
Life Table Test (e)			P=0.001N
Incidental Tumor Test (e)			P=0.001N
Fisher Exact Test (e)			P<0.001N
Pituitary Gland: Adenoma or Carcinoma			
Overall Rates (a)	32/50 (64%)	(b) 16/25 (64%)	14/49 (29%)
Adjusted Rates (c)	70.9%		35.8%
Terminal Rates (d)	23/36 (64%)		12/37 (32%)
Week of First Observation	61		102
Life Table Test (e)			P<0.001N
Incidental Tumor Test (e)			P<0.001N
Fisher Exact Test (e)			P<0.001N
Adrenal Gland: Pheochromocytoma			
Overall Rates (a)	4/50 (8%)	(b) 2/7 (29%)	1/50 (2%)
Adjusted Rates (c)	11.1%		2.6%
Terminal Rates (d)	4/36 (11%)		1/38 (3%)
Week of First Observation	104		104
Life Table Test (e)			P=0.163N
Incidental Tumor Test (e)			P=0.163N
Fisher Exact Test (e)			P=0.181N
Thyroid Gland: C-Cell Adenoma			
Overall Rates (a)	17/50 (34%)	11/49 (22%)	1/50 (2%)
Adjusted Rates (c)	43.4%	25.0%	2.4%
Terminal Rates (d)	14/36 (39%)	11/44 (25%)	0/38 (0%)
Week of First Observation	87	104	102
Life Table Tests (e)	P<0.001N	P=0.043N	P<0.001N
Incidental Tumor Tests (e)	P<0.001N	P=0.078N	P<0.001N
Cochran-Armitage Trend Test (e)	P<0.001N		
Fisher Exact Test (e)		P=0.146N	P<0.001N
Thyroid Gland: C-Cell Carcinoma			
Overall Rates (a)	3/50 (6%)	0/49 (0%)	0/50 (0%)
Adjusted Rates (c)	8.3%	0.0%	0.0%
Terminal Rates (d)	3/36 (8%)	0/44 (0%)	0/38 (0%)
Week of First Observation	104		
Life Table Tests (e)	P=0.030N	P=0.068N	P=0.111N
Incidental Tumor Tests (e)	P=0.030N	P=0.068N	P=0.111N
Cochran-Armitage Trend Test (e)	P=0.038N		
Fisher Exact Test (e)		P=0.125N	P=0.121N

TABLE B3. ANALYSES OF PRIMARY TUMORS IN FEMALE RATS IN THE TWO-YEAR FEED STUDY OF *N*-PHENYL-2-NAPHTHYLAMINE (Continued)

	Control	2,500 ppm	5,000 ppm
Thyroid Gland: C-Cell Adenoma or Carcinoma			
Overall Rates (a)	19/50 (38%)	11/49 (22%)	1/50 (2%)
Adjusted Rates (c)	48.5%	25.0%	2.4%
Terminal Rates (d)	16/36 (44%)	11/44 (25%)	0/38 (0%)
Week of First Observation	87	104	102
Life Table Tests (e)	P<0.001N	P=0.015N	P<0.001N
Incidental Tumor Tests (e)	P<0.001N	P=0.030N	P<0.001N
Cochran-Armitage Trend Test (e)	P<0.001N		
Fisher Exact Test (e)		P=0.071N	P<0.001N
Mammary Gland: Fibroadenoma			
Overall Rates (a)	16/50 (32%)	5/50 (10%)	5/50 (10%)
Adjusted Rates (c)	39.2%	11.0%	12.3%
Terminal Rates (d)	12/36 (33%)	4/44 (9%)	3/38 (8%)
Week of First Observation	84	84	92
Life Table Tests (e)	P=0.003N	P=0.003N	P=0.007N
Incidental Tumor Tests (e)	P=0.002N	P=0.006N	P=0.004N
Cochran-Armitage Trend Test (e)	P=0.003N		
Fisher Exact Test (e)		P=0.007N	P=0.007N
Uterus: Endometrial Stromal Polyp			
Overall Rates (a)	6/50 (12%)	(b) 7/17 (41%)	7/50 (14%)
Adjusted Rates (c)	15.1%		17.1%
Terminal Rates (d)	3/36 (8%)		5/38 (13%)
Week of First Observation	87		88
Life Table Test (e)			P=0.537
Incidental Tumor Test (e)			P=0.531
Fisher Exact Test (e)			P=0.500
All Sites: Benign Tumors			
Overall Rates (a)	42/50 (84%)	35/50 (70%)	26/50 (52%)
Adjusted Rates (c)	89.3%	71.4%	59.0%
Terminal Rates (d)	31/36 (86%)	30/44 (68%)	20/38 (53%)
Week of First Observation	61	69	88
Life Table Tests (e)	P<0.001N	P=0.010N	P=0.010N
Incidental Tumor Tests (e)	P<0.001N	P=0.060N	P<0.001N
Cochran-Armitage Trend Test (e)	P<0.001N		
Fisher Exact Test (e)		P=0.077N	P<0.001N
All Sites: Malignant Tumors			
Overall Rates (a)	22/50 (44%)	8/50 (16%)	10/50 (20%)
Adjusted Rates (c)	48.6%	17.4%	21.9%
Terminal Rates (d)	13/36 (36%)	6/44 (14%)	4/38 (11%)
Week of First Observation	38	91	82
Life Table Tests (e)	P=0.006N	P=0.001N	P=0.014N
Incidental Tumor Tests (e)	P=0.005N	P=0.011N	P=0.008N
Cochran-Armitage Trend Test (e)	P=0.005N		
Fisher Exact Test (e)		P=0.002N	P=0.009N
All Sites: All Tumors			
Overall Rates (a)	46/50 (92%)	38/50 (76%)	33/50 (66%)
Adjusted Rates (c)	93.9%	76.0%	65.7%
Terminal Rates (d)	33/36 (92%)	32/44 (73%)	23/38 (61%)
Week of First Observation	38	69	82
Life Table Tests (e)	P=0.006N	P=0.005N	P=0.011N
Incidental Tumor Tests (e)	P=0.001N	P=0.054N	P=0.001N
Cochran-Armitage Trend Test (e)	P=0.001N		
Fisher Exact Test (e)		P=0.028N	P=0.002N

TABLE B3. ANALYSIS OF PRIMARY TUMORS IN FEMALE RATS IN THE TWO-YEAR FEED STUDY OF *N*-PHENYL-2-NAPHTHYLAMINE (Continued)

(a) Number of tumor-bearing animals/number of animals examined at the site
(b) Incomplete sampling of tissues
(c) Kaplan-Meier estimated tumor incidence at the end of the study after adjusting for intercurrent mortality
(d) Observed tumor incidence at terminal kill
(e) Beneath the control incidence are the P values associated with the trend test. Beneath the dosed group incidence are the P values corresponding to pairwise comparisons between that dosed group and the controls. The life table analysis regards tumors in animals dying prior to terminal kill as being (directly or indirectly) the cause of death. The incidental tumor test regards these lesions as incidental. The Cochran-Armitage and Fisher exact tests compare directly the overall incidence rates. A negative trend or lower incidence in a dosed group is indicated by (N).
(f) Only three spleens, three lymph nodes, three thymuses, three bone marrow samples, and five small intestines were examined.

TABLE B4a. HISTORICAL INCIDENCE OF THYROID GLAND C-CELL TUMORS IN FEMALE F344/N RATS RECEIVING NO TREATMENT (a)

Study	Incidence in Controls		
	Adenoma	Carcinoma	Adenoma or Carcinoma
Historical Incidence at Battelle Columbus Laboratories			
Chlordecone	0/49	2/49	2/49
Common control group (b)	0/50	2/50	2/50
C.I. Disperse Yellow 3	0/49	1/49	1/49
D&C Red No. 9	2/47	3/47	5/47
C.I. Solvent Yellow 14	0/50	2/50	2/50
l-Ascorbic acid	2/49	0/49	2/49
TOTAL	4/330 (1.2%)	12/330 (3.6%)	16/330 (4.8%)
SD (c)	2.15%	2.45%	3.03%
Range (d)			
High	2/47	3/47	5/47
Low	0/50	0/49	1/49
Overall Historical Incidence			
TOTAL	114/1,952 (5.8%)	71/1,952 (3.6%)	182/1,952 (9.3%)
SD (c)	5.02%	2.59%	5.49%
Range (d)			
High	9/50	5/50	11/50
Low	0/50	0/50	0/50

(a) Data as of August 30, 1988, for studies of at least 104 weeks
(b) Ninety-animal common control group for the C.I. Acid Orange 10, FD&C Yellow No. 6, and C.I. Acid Red 14 studies
(c) Standard deviation
(d) Range and SD are presented for groups of 35 or more animals.

TABLE B4b. HISTORICAL INCIDENCE OF MAMMARY GLAND TUMORS IN FEMALE F344/N RATS RECEIVING NO TREATMENT (a)

Study	Incidence of Fibroadenomas in Controls
Historical Incidence at Battelle Columbus Laboratories	
Chlorobenzene	7/49
Common control group (b)	23/98
C.I. Disperse Yellow 3	7/50
D&C Red No. 9	10/50
C.I. Solvent Yellow 14	7/50
l-Ascorbic Acid	5/50
TOTAL	59/327 (17.3%)
SD (c)	5.30%
Range (d)	
High	23/98
Low	5/50
Overall Historical Incidence	
TOTAL	(e) 563/2,021 (27.9%)
SD (c)	11.69%
Range (d)	
High	34/49
Low	5/50

(a) Data as of August 30, 1988, for studies of at least 104 weeks
(b) Ninety-animal common control group for the C.I. Acid Orange 10, FD&C Yellow No. 6, and C.I. Acid Red 14 studies
(c) Standard deviation
(d) Range and SD are presented for groups of 35 or more animals.
(e) Includes four diagnoses of cystifibroadenoma

TABLE B4c. HISTORICAL INCIDENCE OF LEUKEMIA IN FEMALE F344/N RATS RECEIVING NO TREATMENT (a)

Study	Incidence in Controls
Historical Incidence at Battelle Columbus Laboratories	
Chlorobenzene	3/49
Common control group (b)	10/98
C.I. Disperse Yellow 3	3/49
D&C Red No. 9	10/98
C.I. Solvent Yellow 14	3/49
l-Ascorbic Acid	6/50
TOTAL	55/327 (17.2%)
SD (c)	2.59%
Range (d)	
High	10/50
Low	0/50
Overall Historical Incidence	
TOTAL	375/2,021 (18.6%)
SD (c)	6.59%
Range (d)	
High	10/50
Low	3/50

(a) Data as of August 30, 1988, for studies of at least 104 weeks
(b) Ninety-animal common control group for the C.I. Acid Orange 10, FD&C Yellow No. 6, and C.I. Acid Red 14 studies
(c) Standard deviation
(d) Range and SD are presented for groups of 35 or more animals.

TABLE B44. HISTORICAL INCIDENCE OF PITUITARY GLAND TUMORS IN FEMALE F344/N RATS RECEIVING NO TREATMENT (a)

Study	Incidence in Controls		
	Adenoma (b)	Carcinoma (c)	Adenoma or Carcinoma (b,c)
Historical Incidence at Battelle Columbus Laboratories			
Chlorobenzene	27/48	1/48	28/48
Common control group (d)	26/88	5/88	30/88
C.I. Disperse Yellow 3	15/44	1/44	16/44
D&C Red No. 9	21/48	2/48	23/48
C.I. Solvent Yellow 14	28/44	0/44	28/44
l-Ascorbic Acid	28/50	1/50	29/50
TOTAL	141/312 (45.2%)	10/312 (3.2%)	151/312 (48.4%)
SD (e)	12.56%	2.15%	11.40%
Range (f)			
High	28/44	5/88	28/44
Low	15/88	0/44	29/88
Overall Historical Incidence			
TOTAL	862/1,962 (44.2%)	71/1,962 (3.6%)	931/1,962 (47.7%)
SD (e)	11.56%	3.97%	11.02%
Range (f)			
High	33/47	8/49	33/47
Low	7/39	0/50	9/39

(a) Data as of August 26, 1985, for studies of at least 104 weeks
(b) Includes all adenomas diagnosed as NOS, chromophobe, or acidophil
(c) Includes adenocarcinomas, NOS, and carcinomas diagnosed as NOS and chromophobe
(d) Ninety-animal common control group for the C.I. Acid Orange 10, FD&C Yellow No. 6, and C.I. Acid Red 14 studies
(e) Standard deviation
(f) Range and SD are presented for groups of 35 or more animals.

TABLE B5. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE RATS IN THE TWO-YEAR FEED STUDY OF *N*-PHENYL-2-NAPHTHYLAMINE

	Untreated Control	Low Dose	High Dose
ANIMALS INITIALLY IN STUDY	50	50	50
ANIMALS NECROPSIED	50	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY	50	50	50
INTEGUMENTARY SYSTEM			
None			
RESPIRATORY SYSTEM			
*Nasal cavity	(50)	(50)	(50)
Foreign body, NOS			1 (2%)
*Nasal turbinate	(50)	(50)	(50)
Inflammation, acute suppurative			1 (2%)
Inflammation, acute/chronic	18 (36%)	1 (2%)	15 (30%)
#Lung	(50)	(4)	(50)
Inflammation, chronic	1 (2%)		1 (2%)
Hyperplasia, epithelial			3 (6%)
HEMATOPOIETIC SYSTEM			
#Bone marrow	(50)	(3)	(49)
Myelofibrosis			2 (4%)
Hyperplasia, reticulum cell	1 (2%)	1 (33%)	
#Spleen	(49)	(3)	(50)
Inflammation, acute/chronic	1 (2%)		
#Splenic capsule	(49)	(3)	(50)
Fibrosis, multifocal	1 (2%)		
#Splenic follicles	(49)	(3)	(50)
Depletion, lymphoid			3 (6%)
Hyperplasia, reticulum cell	1 (2%)		
#Splenic red pulp	(49)	(3)	(50)
Fibrosis	2 (4%)		
Hemosiderosis			4 (8%)
#Mandibular lymph node	(49)	(3)	(48)
Inflammation, acute focal			1 (2%)
#Mesenteric lymph node	(49)	(3)	(48)
Inflammation, chronic diffuse			1 (2%)
#Renal lymph node	(49)	(3)	(48)
Edema, NOS			1 (2%)
#Liver	(50)	(50)	(50)
Hematopoiesis		1 (2%)	
#Thymus	(50)	(3)	(42)
Depletion, lymphoid	44 (88%)		38 (90%)
CIRCULATORY SYSTEM			
#Myocardium	(50)	(4)	(50)
Degeneration, NOS	48 (96%)	3 (75%)	46 (92%)
*Coronary artery	(50)	(50)	(50)
Inflammation, acute/chronic	1 (2%)		
*Hepatic artery	(50)	(50)	(50)
Dilatation, NOS	1 (2%)		
*Suppurative pancreaticoduodenal artery	(50)	(50)	(50)
Inflammation, acute/chronic	2 (4%)		
Inflammation, chronic			1 (2%)
#Liver	(50)	(50)	(50)
Thrombus, mural		1 (2%)	

TABLE B6. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE RATS IN THE TWO-YEAR FEED STUDY OF *N*-PHENYL-2-NAPHTHYLAMINE (Continued)

	Untreated Control	Low Dose	High Dose
DIGESTIVE SYSTEM			
#Salivary gland	(48)	(4)	(48)
Focal cellular change			1 (2%)
#Liver	(50)	(50)	(50)
Hernia, NOS	3 (6%)	3 (6%)	2 (4%)
Inflammation, acute/chronic	28 (56%)	13 (26%)	1 (2%)
Degeneration, cystic	6 (12%)	1 (2%)	2 (4%)
Necrosis, NOS	2 (4%)	3 (6%)	
Basophilic cyte change	45 (90%)	48 (96%)	46 (92%)
Eosinophilic cyte change	1 (2%)		
Clear cell change	5 (10%)	3 (6%)	2 (4%)
Hyperplasia, focal		1 (2%)	
Angiectasis	1 (2%)	1 (2%)	1 (2%)
Regeneration, NOS	2 (4%)		
#Hepatic capsule	(50)	(50)	(50)
Fibrosis, focal	1 (2%)		
#Liver/centrilobular	(50)	(50)	(50)
Cytoplasmic vacuolization	2 (4%)		
#Liver/hepatocytes	(50)	(50)	(50)
Hypertrophy, focal	1 (2%)		
#Bile duct	(50)	(50)	(50)
Hyperplasia, NOS	18 (36%)	8 (16%)	10 (20%)
#Pancreas	(50)	(4)	(50)
Dilatation/ducts	1 (2%)		
#Pancreatic acinus	(50)	(4)	(50)
Atrophy, focal	12 (24%)	1 (25%)	6 (12%)
#Esophagus	(50)	(4)	(50)
Inflammation, acute necrotizing			1 (2%)
#Perianopharyngeal tissue	(50)	(4)	(50)
Inflammation, chronic focal			1 (2%)
#Glandular stomach	(50)	(3)	(50)
Cyst, NOS	1 (2%)		
Inflammation, acute/chronic	1 (2%)		
Necrosis, focal			2 (4%)
#Forestomach	(50)	(3)	(50)
Inflammation, acute/chronic	1 (2%)		
#Jejunum	(49)	(5)	(49)
Diverticulum		1 (20%)	
#Colon	(48)	(4)	(48)
Parasitism	4 (8%)		1 (2%)
#Cecum	(49)	(4)	(48)
Mineralization			1 (2%)
Edema, NOS			1 (2%)
Inflammation, chronic focal			1 (2%)
URINARY SYSTEM			
#Kidney	(50)	(50)	(50)
Mineralization	9 (18%)	20 (40%)	23 (46%)
Hydronephrosis		1 (2%)	47 (94%)
Cyst, NOS			4 (8%)
Pyelonephritis, acute		1 (2%)	
Inflammation, chronic focal		4 (8%)	41 (82%)
Nephropathy	46 (92%)	43 (86%)	50 (100%)
Atrophy, NOS		1 (2%)	22 (44%)
Hyperplasia, tubular cell	1 (2%)		1 (2%)
#Kidney/interstitium	(50)	(50)	(50)
Fibrosis, multifocal			43 (86%)
#Kidney/medulla	(50)	(50)	(50)
Mineralization		1 (2%)	
Pyelonephritis, acute	1 (2%)		

TABLE B6. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE RATS IN THE TWO-YEAR FEED STUDY OF *N*-PHENYL-2-NAPHTHYLAMINE (Continued)

	Untreated Control	Low Dose	High Dose
URINARY SYSTEM (Continued)			
#Renal papilla	(50)	(50)	(50)
Necrosis, NOS		7 (14%)	9 (18%)
#Kidney/tubule	(50)	(50)	(50)
Inflammation, acute suppurative	2 (4%)	4 (8%)	23 (46%)
#Kidney/pelvis	(50)	(50)	(50)
Calculus, microscopic examination		12 (24%)	11 (22%)
Hemorrhage	1 (2%)		
Inflammation, chronic focal			2 (4%)
Hyperplasia, epithelial	2 (4%)	12 (24%)	49 (98%)
#Urinary bladder	(49)	(3)	(50)
Hemorrhage	1 (2%)		
Inflammation, acute/chronic	1 (2%)		
Necrosis, hemorrhagic	1 (2%)		
Hyperplasia, papillary	1 (2%)		
ENDOCRINE SYSTEM			
#Pituitary intermedia	(50)	(25)	(49)
Multiple cysts		1 (4%)	
#Anterior pituitary	(50)	(25)	(49)
Mineralization			1 (2%)
Cyst, NOS	1 (2%)	4 (16%)	5 (10%)
Multiple cysts	20 (40%)	8 (32%)	13 (27%)
Hemorrhage	1 (2%)		
Hyperplasia, NOS	11 (22%)	5 (20%)	14 (29%)
#Area cava/capsule	(50)	(7)	(50)
Hyperplasia, focal			1 (2%)
#Adrenal cortex	(50)	(7)	(50)
Cyst, NOS	1 (2%)		
Necrosis, focal	2 (4%)		
Metamorphosis, fatty	3 (6%)	1 (14%)	1 (2%)
Atrophy, diffuse	1 (2%)		
Hypertrophy, NOS	1 (2%)		
Hypertrophy, focal	4 (8%)	1 (14%)	5 (10%)
Hyperplasia, NOS	25 (50%)	2 (29%)	26 (52%)
#Adrenal medulla	(50)	(7)	(50)
Hyperplasia, NOS	3 (6%)		6 (12%)
#Thyroid	(50)	(49)	(50)
Follicular cyst, NOS		2 (4%)	1 (2%)
Hyperplasia, C-cell	43 (86%)	41 (84%)	43 (86%)
#Parathyroid	(38)	(44)	(41)
Hyperplasia, NOS	1 (3%)	2 (5%)	3 (7%)
Hyperplasia, diffuse			1 (2%)
REPRODUCTIVE SYSTEM			
*Mammary gland	(50)	(50)	(50)
Hyperplasia, cystic	42 (84%)	2 (4%)	41 (82%)
*Clitoral gland	(50)	(50)	(50)
Dilatation/ducts	1 (2%)	1 (2%)	
Inflammation, acute suppurative	1 (2%)		
Inflammation, acute/chronic	30 (60%)		21 (42%)
Hyperplasia, focal	1 (2%)		
Hyperkeratosis			1 (2%)
Acanthosis			1 (2%)
#Uterus	(50)	(17)	(50)
Dilatation, NOS	5 (10%)		7 (14%)
Hemorrhage			1 (2%)
Inflammation, acute suppurative	1 (2%)		

TABLE B6. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE RATS IN THE TWO-YEAR FEED STUDY OF *N*-PHENYL-2-NAPHTHYLAMINE (Continued)

	Untreated Control	Low Dose	High Dose
REPRODUCTIVE SYSTEM (Continued)			
#Cervix uteri	(50)	(17)	(50)
Dilatation, NOS	1 (2%)		
Diverticulum		3 (18%)	
Inflammation, acute suppurative	2 (4%)		
Fibrosis, multifocal	1 (2%)		
#Uterus/endometrium	(50)	(17)	(50)
Polypoid hyperplasia	1 (2%)		
#Endometrial gland	(50)	(17)	(50)
Hyperplasia, cystic	10 (20%)	5 (29%)	14 (28%)
#Ovary	(50)	(8)	(50)
Follicular cyst, NOS	1 (2%)	1 (13%)	5 (10%)
Parovarian cyst	6 (12%)	3 (38%)	4 (8%)
NERVOUS SYSTEM			
#Cerebral ventricle	(50)	(3)	(50)
Hydrocephalus, NOS	2 (4%)		
#Brain	(50)	(3)	(50)
Cyst, NOS	1 (2%)		
Hemorrhage	1 (2%)		
Atrophy, pressure	11 (22%)	1 (33%)	2 (4%)
SPECIAL SENSE ORGANS			
*Eyelcrystalline lens	(50)	(50)	(50)
Cataract			1 (2%)
*Harderian gland	(50)	(50)	(50)
Hyperplasia, epithelial	1 (2%)		
MUSCULOSKELETAL SYSTEM			
*Femur	(50)	(50)	(50)
Hyperostosis			1 (2%)
BODY CAVITIES			
None			
ALL OTHER SYSTEMS			
None			
SPECIAL MORPHOLOGY SUMMARY			
None			

* Number of animals receiving complete necropsy examinations; all gross lesions including masses examined microscopically.
# Number of animals examined microscopically at this site

APPENDIX C

SUMMARY OF LESIONS IN MALE MICE IN THE TWO-YEAR FEED STUDY OF *N*-PHENYL-2-NAPTHYLAMINE

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TABLE C1. SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MALE MICE IN THE TWO-YEAR FEED STUDY OF *N*-PHENYL-2-NAPHTHYLAMINE

	Untreated Control	Low Dose	High Dose
ANIMALS INITIALLY IN STUDY	50	50	50
ANIMALS MISSING	1		
ANIMALS NECROPSIED	49	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY	49	50	50
INTEGUMENTARY SYSTEM			
*Skin	(49)	(50)	(50)
Basal cell tumor	1 (2%)		
*Subcutaneous tissue	(49)	(50)	(50)
Sarcoma, NOS		1 (2%)	5 (10%)
Fibroma	2 (4%)	3 (6%)	
Fibrosarcoma	2 (4%)	2 (4%)	†3 (6%)
Osteosarcoma			1 (2%)
Neurofibrosarcoma		1 (2%)	
RESPIRATORY SYSTEM			
#Lung	(49)	(50)	(48)
Hepatocellular carcinoma, metastatic	1 (2%)	2 (4%)	3 (7%)
Alveolar/bronchiolar adenoma	6 (12%)	7 (14%)	5 (11%)
Alveolar/bronchiolar carcinoma	5 (10%)	2 (4%)	2 (4%)
Fibrosarcoma, metastatic	1 (2%)		
HEMATOPOIETIC SYSTEM			
*Multiple organs	(49)	(50)	(50)
Malignant lymphoma, undiffer type		1 (2%)	
Malignant lymphoma, lymphocytic type	2 (4%)	1 (2%)	2 (4%)
Malignant lymphoma, histiocytic type	3 (6%)		2 (4%)
Malignant lymphoma, mixed type			2 (4%)
#Lumbar lymph node	(44)	(22)	(44)
Sarcoma, NOS, metastatic			1 (2%)
#Mesenteric lymph node	(44)	(22)	(44)
Malignant lymphoma, lymphocytic type		1 (5%)	
CIRCULATORY SYSTEM			
*Multiple organs	(49)	(50)	(50)
Hemangiosarcoma	2 (4%)		
#Spleen	(45)	(16)	(48)
Hemangiosarcoma	1 (2%)		1 (2%)
#Liver	(47)	(50)	(47)
Hemangiosarcoma	2 (4%)		
DIGESTIVE SYSTEM			
#Liver	(47)	(50)	(47)
Hepatocellular adenoma	6 (13%)	12 (24%)	10 (21%)
Hepatocellular carcinoma	6 (13%)	5 (10%)	9 (19%)
Mixed hepatocholangio carcinoma		1 (2%)	
URINARY SYSTEM			
#Kidney	(49)	(50)	(47)
Hepatocellular carcinoma, metastatic	1 (2%)	1 (2%)	
Tubular cell adenocarcinoma	1 (2%)		

TABLE C1. SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MALE MICE IN THE TWO-YEAR FEED STUDY OF *N*-PHENYL-2-NAPHTHYLAMINE (Continued)

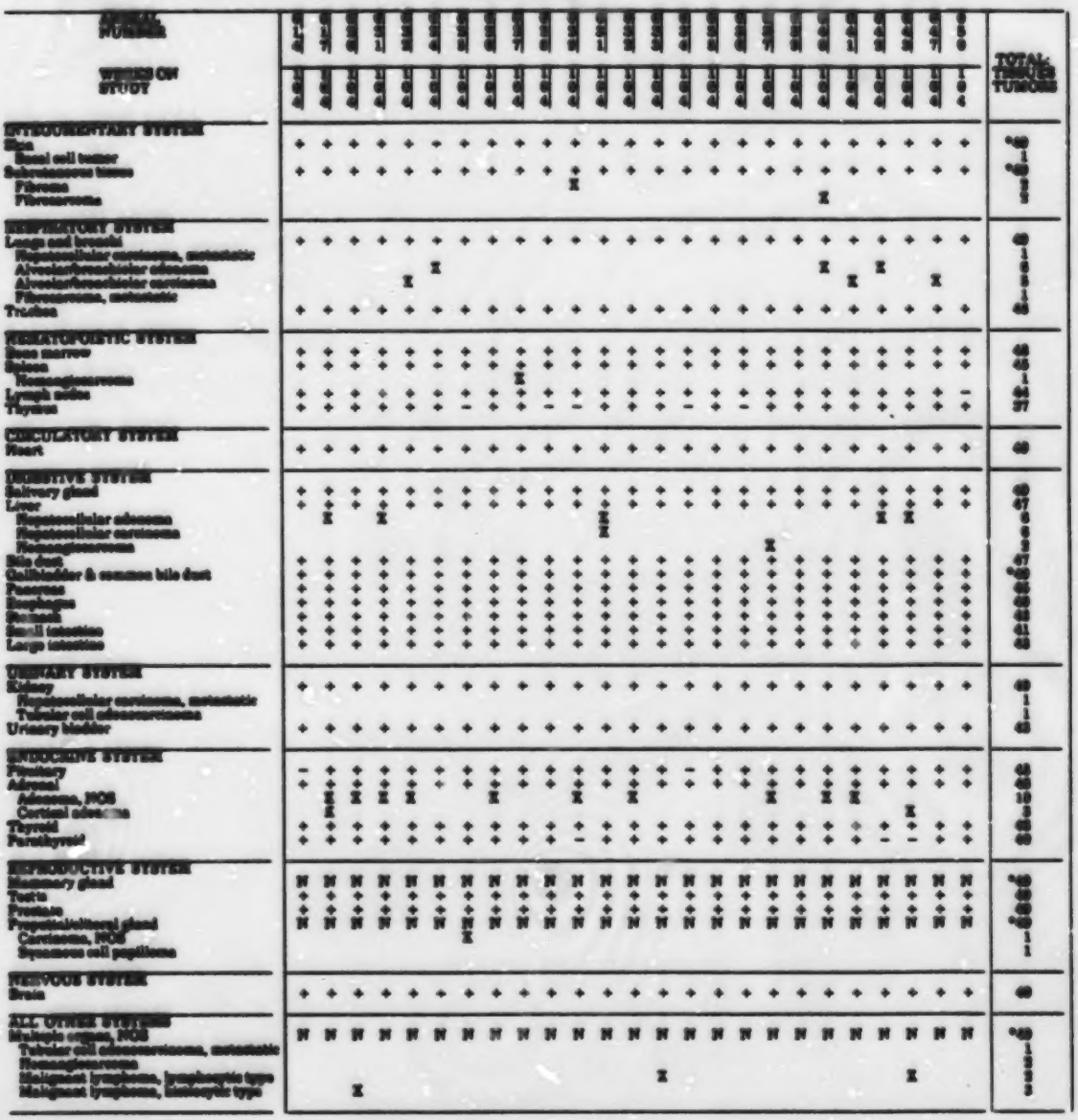
	Untreated Control	Low Dose	High Dose
ENDOCRINE SYSTEM			
#Adrenal	(48)	(12)	(46)
Cortical adenoma	3 (6%)		1 (2%)
#Adrenal/capsule	(48)	(12)	(46)
Adenoma, NOS	10 (21%)		6 (13%)
#Adrenal medulla	(48)	(12)	(46)
Pheochromocytoma		1 (8%)	
REPRODUCTIVE SYSTEM			
*Preputial gland	(49)	(50)	(50)
Carcinoma, NOS	1 (2%)		
Squamous cell papilloma	1 (2%)		
NERVOUS SYSTEM			
None			
SPECIAL SENSE ORGANS			
*Harderian gland	(49)	(50)	(50)
Adenoma, NOS		2 (4%)	2 (4%)
MUSCULOSKELETAL SYSTEM			
*Femur	(49)	(50)	(50)
Osteoma			1 (2%)
BODY CAVITIES			
None			
ALL OTHER SYSTEMS			
*Multiple organs	(49)	(50)	(50)
Mixed hepato/cholangiocarcinoma, metastatic		1 (2%)	
Tubular cell adenocarcinoma, metastatic	1 (2%)		
ANIMAL DISPOSITION SUMMARY			
Animals initially in study	50	50	50
Natural death	13	8	18
Moribund sacrifice	3	6	4
Terminal sacrifice	33	36	28
Animal missing	1		
TUMOR SUMMARY			
Total animals with primary tumors**	34	30	32
Total primary tumors	54	40	53
Total animals with benign tumors	19	20	19
Total benign tumors	29	25	25
Total animals with malignant tumors	23	14	23
Total malignant tumors	25	15	28
Total animals with secondary tumors##	3	3	4
Total secondary tumors	4	4	4

* Number of animals receiving complete necropsy examinations; all gross lesions including masses examined microscopically.
† Multiple occurrence of morphology in the same organ. Tissue is counted once only.
** Primary tumors: all tumors except secondary tumors
# Number of animals examined microscopically at this site
Secondary tumors: metastatic tumors or tumors invasive into an adjacent organ

TABLE C2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF MALE MICE IN THE TWO-YEAR FEED STUDY OF *N*-PHENYL-2-NAPHTHYLAMINE: UNTREATED CONTROL

ANIMAL NUMBER	0 0 4	0 1 0	0 0 6	0 1 1	0 0 8	0 0 9	0 1 2	0 0 3	0 0 5	0 1 0	0 0 7	0 0 8	0 0 9	0 1 1	0 0 1	0 0 2	0 0 3	0 0 5	0 0 7	0 0 8	0 0 9	0 1 0	0 1 2	0 1 3
WEEKS ON STUDY	0 3 7	0 3 9	0 3 9	0 3 9	0 3 9	0 3 9	0 3 9	0 3 9	0 3 9	0 3 9	0 3 9	0 3 9	0 3 9	0 3 9	0 4 0	0 4 0	0 4 0	0 4 0	0 4 0	0 4 0	0 4 0	0 4 0	0 4 0	1 0 4
INTEGUMENTARY SYSTEM																								
Skin	+	M	+	+	+	+	+	+	+	+	+	N	+	+	+	+	+	+	+	+	+	+	+	+
Basal cell tumor																X								
Subcutaneous tissue	+	M	+	+	+	+	+	+	+	+	+	N	+	+	+	+	+	+	+	+	+	+	+	+
Fibroma																X								
Fibrosarcoma										X														
RESPIRATORY SYSTEM																								
Lungs and bronchi	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatocellular carcinoma, metastatic															X									
Alveolar/bronchiolar adenoma																X				X				X
Alveolar/bronchiolar carcinoma				X																	X			
Fibrosarcoma, metastatic										X														
Trachea	+	M	+	+	+	+	+	+	+	+	+	+	+	–	+	+	+	+	+	+	+	+	+	+
HEMATOPOIETIC SYSTEM																								
Bone marrow	+	M	+	+	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spleen	+	M	–	+	–	–	+	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hemangiosarcoma																								
Lymph nodes	–	M	+	–	+	+	+	–	+	+	+	–	+	+	+	+	+	+	+	+	+	+	+	+
Thymus	+	M	+	–	+	+	+	–	+	+	+	–	+	+	–	+	–	+	–	+	+	+	–	+
CIRCULATORY SYSTEM																								
Heart	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
DIGESTIVE SYSTEM																								
Salivary gland	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Liver	+	M	+	–	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatocellular adenoma																			X					
Hepatocellular carcinoma												X	X	X	X							X		
Hemangiosarcoma								X																
Bile duct	+	M	+	–	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Gallbladder & common bile duct	+	M	+	N	N	N	N	N	N	N	+	+	+	+	N	N	+	+	+	+	+	+	+	+
Pancreas	+	M	+	–	–	–	+	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Esophagus	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach	+	M	–	+	–	+	–	–	+	+	+	–	+	+	+	+	+	+	+	+	+	+	–	+
Small intestine	+	M	–	–	–	–	–	–	+	+	+	+	+	–	+	+	+	+	+	+	+	+	+	+
Large intestine	+	M	–	–	–	+	–	–	+	+	+	+	+	–	+	+	+	+	+	+	+	+	+	+
URINARY SYSTEM																								
Kidney	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatocellular carcinoma, metastatic															X									
Tubular cell adenocarcinoma													X											
Urinary bladder	+	M	+	–	–	–	+	–	+	+	+	–	+	+	+	+	+	–	+	+	+	+	+	+
ENDOCRINE SYSTEM																								
Pituitary	–	M	+	+	+	–	+	+	+	+	+	–	+	+	+	+	–	+	+	+	+	+	+	+
Adrenal	+	M	+	+	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma, NOS																								
Cortical adenoma																			X					
Thyroid	+	M	+	+	+	+	+	+	+	+	+	+	+	–	+	+	+	+	+	+	+	+	+	+
Parathyroid	+	M	–	+	–	+	–	+	+	+	+	–	+	–	+	+	+	+	+	+	–	+	+	+
REPRODUCTIVE SYSTEM																								
Mammary gland	N	M	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Testis	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Prostate	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Preputial/clitoral gland	N	M	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Carcinoma, NOS																								
Squamous cell papilloma						X																		
NERVOUS SYSTEM																								
Brain	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
ALL OTHER SYSTEMS																								
Multiple organs, NOS	N	M	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Tubular cell adenocarcinoma, metastatic													X											
Hemangiosarcoma							X							X										
Malignant lymphoma, lymphocytic type										X														
Malignant lymphoma, histiocytic type																X								

TABLE C2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF MALE MICE: UNTREATED CONTROL (Continued)



* Animals necropsied

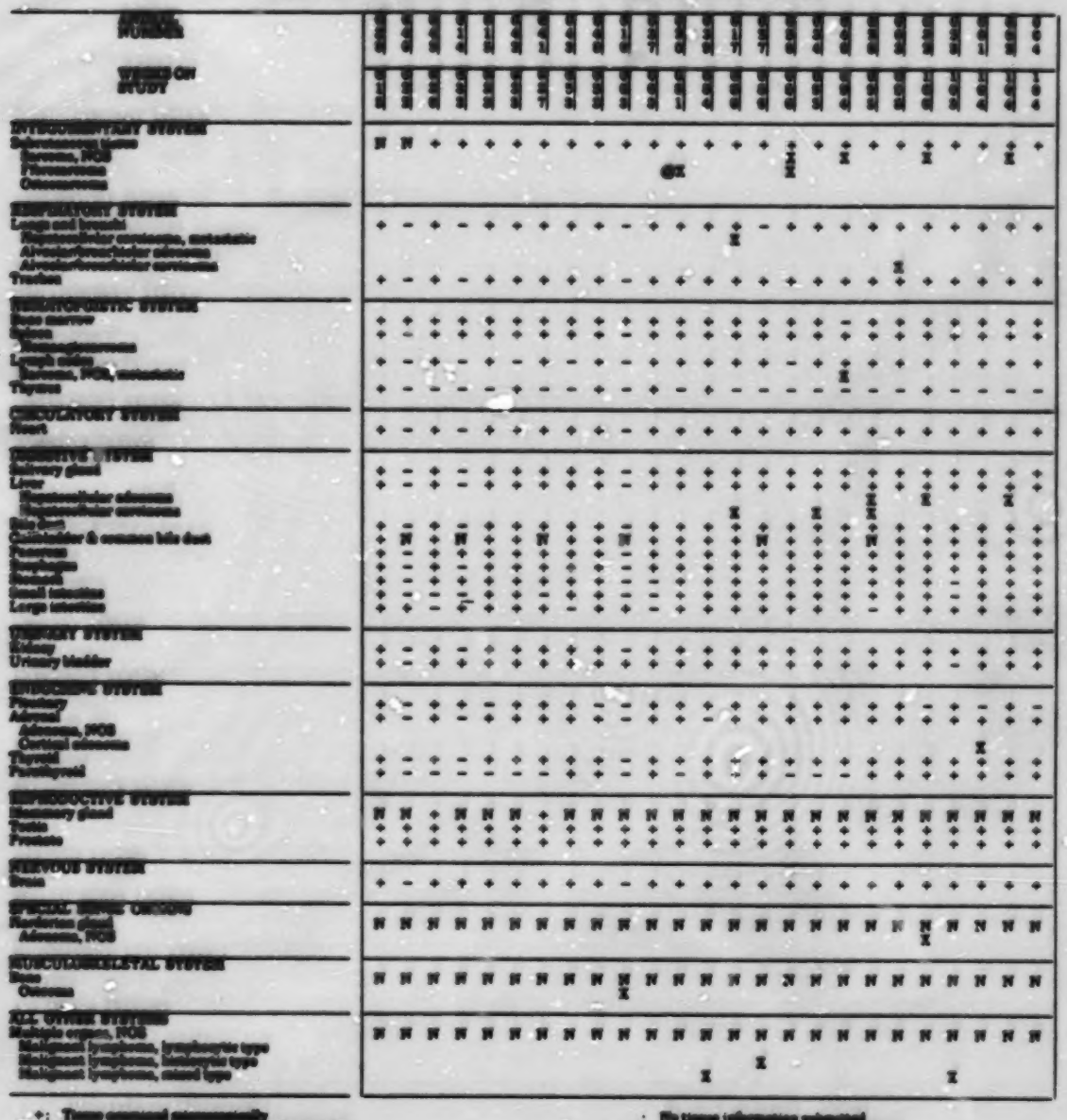
TABLE C2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF MALE MICE IN THE TWO-YEAR FEED STUDY OF *N*-PHENYL-2-NAPHTHYLAMINE: LOW DOSE

| ANIMAL NUMBER | 0 0 0

TABLE C2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF MALE MICE: LOW DOSE
(Continued)

| ANIMAL NUMBER | | |

TABLE C2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF MALE MICE IN THE TWO-YEAR FEED STUDY OF *N*-PHENYL-2-NAPHTHYLAMINE: HIGH DOSE



+: Tissue examined microscopically
-: Required tissue not examined microscopically
X: Tumor incidence
B: Necropsy, no autolysis, no microscopic examination
A: Animal missing
N: No necropsy performed
[#]: Multiple occurrence of morphology

.: No tissue information submitted
C: Necropsy, no histology due to protocol
A: Autolysis
M: Animal missing

TABLE C2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF MALE MICE: HIGH DOSE
(Continued)

| ANIMAL NUMBER | | |

TABLE C3. ANALYSIS OF PRIMARY TUMORS IN MALE MICE IN THE TWO-YEAR FEED STUDY OF *N*-PHENYL-2-NAPHTHYLAMINE

	Control	2,500 ppm	5,000 ppm
Subcutaneous Tissue: Fibroma			
Overall Rates (a)	2/49 (4%)	3/50 (6%)	0/50 (0%)
Adjusted Rates (b)	5.8%	8.3%	0.0%
Terminal Rates (c)	1/33 (3%)	3/36 (8%)	0/28 (0%)
Week of First Observation	100	104	
Life Table Tests (d)	P=0.238N	P=0.539	P=0.275N
Incidental Tumor Tests (d)	P=0.191N	P=0.532	P=0.181N
Cochran-Armitage Trend Test (d)	P=0.196N		
Fisher Exact Test (d)		P=0.510	P=0.242N
Subcutaneous Tissue: Fibrosarcoma			
Overall Rates (a)	2/49 (4%)	2/50 (4%)	3/50 (6%)
Adjusted Rates (b)	5.5%	5.0%	8.8%
Terminal Rates (c)	1/33 (3%)	0/36 (0%)	1/28 (4%)
Week of First Observation	86	87	81
Life Table Tests (d)	P=0.363	P=0.665N	P=0.456
Incidental Tumor Tests (d)	P=0.480	P=0.613	P=0.531
Cochran-Armitage Trend Test (d)	P=0.415		
Fisher Exact Test (d)		P=0.684N	P=0.510
Subcutaneous Tissue: Sarcoma, Fibrosarcoma, or Neurofibrosarcoma			
Overall Rates (a)	2/49 (4%)	4/50 (8%)	8/50 (16%)
Adjusted Rates (b)	5.5%	9.6%	23.6%
Terminal Rates (c)	1/33 (3%)	0/36 (0%)	3/28 (11%)
Week of First Observation	86	79	81
Life Table Tests (d)	P=0.022	P=0.272	P=0.027
Incidental Tumor Tests (d)	P=0.048	P=0.213	P=0.064
Cochran-Armitage Trend Test (d)	P=0.031		
Fisher Exact Test (d)		P=0.349	P=0.049
Subcutaneous Tissue: Fibroma or Fibrosarcoma			
Overall Rates (a)	4/49 (8%)	5/50 (10%)	3/50 (6%)
Adjusted Rates (b)	11.0%	12.9%	8.8%
Terminal Rates (c)	2/33 (6%)	3/36 (8%)	1/28 (4%)
Week of First Observation	86	87	81
Life Table Tests (d)	P=0.498N	P=0.549	P=0.561N
Incidental Tumor Tests (d)	P=0.363N	P=0.490	P=0.433N
Cochran-Armitage Trend Test (d)	P=0.415N		
Fisher Exact Test (d)		P=0.513	P=0.489N
Subcutaneous Tissue: Fibroma, Sarcoma, Fibrosarcoma, or Neurofibrosarcoma			
Overall Rates (a)	4/49 (8%)	7/50 (14%)	8/50 (16%)
Adjusted Rates (b)	11.0%	17.2%	23.0%
Terminal Rates (c)	2/33 (6%)	3/36 (8%)	3/28 (11%)
Week of First Observation	86	79	81
Life Table Tests (d)	P=0.107	P=0.314	P=0.137
Incidental Tumor Tests (d)	P=0.216	P=0.193	P=0.247
Cochran-Armitage Trend Test (d)	P=0.185		
Fisher Exact Test (d)		P=0.274	P=0.189
Lung: Alveolar/Bronchiolar Adenoma			
Overall Rates (a)	6/49 (12%)	7/50 (14%)	5/46 (11%)
Adjusted Rates (b)	17.6%	18.8%	17.9%
Terminal Rates (c)	5/33 (15%)	6/36 (17%)	5/28 (18%)
Week of First Observation	100	88	104
Life Table Tests (d)	P=0.552N	P=0.564	P=0.615N
Incidental Tumor Tests (d)	P=0.520N	P=0.527	P=0.563N
Cochran-Armitage Trend Test (d)	P=0.484N		
Fisher Exact Test (d)		P=0.516	P=0.545N

TABLE C3. ANALYSIS OF PRIMARY TUMORS IN MALE MICE IN THE TWO-YEAR FEED STUDY OF *N*-PHENYL-2-NAPHTHYLAMINE (Continued)

	Control	2,500 ppm	5,000 ppm
Lung: Alveolar/Bronchiolar Carcinoma			
Overall Rates (a)	5/49 (10%)	2/50 (4%)	2/46 (4%)
Adjusted Rates (b)	14.0%	5.0%	6.7%
Terminal Rates (c)	4/33 (12%)	1/36 (3%)	1/28 (4%)
Week of First Observation	54	69	98
Life Table Tests (d)	P=0.186N	P=0.194N	P=0.285N
Incidental Tumor Tests (d)	P=0.204N	P=0.268N	P=0.265N
Cochran-Armitage Trend Test (d)	P=0.163N		
Fisher Exact Test (d)		P=0.216N	P=0.245N
Lung: Alveolar/Bronchiolar Adenoma or Carcinoma			
Overall Rates (a)	11/49 (22%)	9/50 (18%)	7/46 (15%)
Adjusted Rates (b)	30.9%	23.2%	24.0%
Terminal Rates (c)	9/33 (27%)	7/36 (19%)	6/28 (21%)
Week of First Observation	54	69	98
Life Table Tests (d)	P=0.280N	P=0.331N	P=0.340N
Incidental Tumor Tests (d)	P=0.271N	P=0.423N	P=0.286N
Cochran-Armitage Trend Test (d)	P=0.219N		
Fisher Exact Test (d)		P=0.382N	P=0.263N
Hematopoietic System: Malignant Lymphoma, Histiocytic Type			
Overall Rates (a)	3/49 (6%)	(e,f) 0/50 (0%)	2/50 (4%)
Adjusted Rates (b)	8.1%		6.2%
Terminal Rates (c)	1/33 (3%)		1/28 (4%)
Week of First Observation	79		86
Life Table Test (d)			P=0.555N
Incidental Tumor Test (d)			P=0.469N
Fisher Exact Test (d)			P=0.491N
Hematopoietic System: Lymphoma, All Malignant			
Overall Rates (a)	5/49 (10%)	(e,f) 3/50 (6%)	6/50 (12%)
Adjusted Rates (b)	13.8%		18.3%
Terminal Rates (c)	3/33 (9%)		3/28 (11%)
Week of First Observation	79		84
Life Table Test (d)			P=0.412
Incidental Tumor Test (d)			P=0.523
Fisher Exact Test (d)			P=0.514
Circulatory System: Hemangiosarcoma			
Overall Rates (a)	5/49 (10%)	(e,f) 0/50 (0%)	1/50 (2%)
Adjusted Rates (b)	12.8%		3.6%
Terminal Rates (c)	2/33 (6%)		1/28 (4%)
Week of First Observation	59		104
Life Table Test (d)			P=0.138N
Incidental Tumor Test (d)			P=0.160N
Fisher Exact Test (d)			P=0.098N
Liver: Hepatocellular Adenoma			
Overall Rates (a)	6/47 (13%)	12/50 (24%)	10/47 (21%)
Adjusted Rates (b)	18.2%	31.3%	33.1%
Terminal Rates (c)	6/33 (18%)	10/36 (28%)	8/28 (29%)
Week of First Observation	104	77	95
Life Table Tests (d)	P=0.096	P=0.134	P=0.116
Incidental Tumor Tests (d)	P=0.136	P=0.121	P=0.162
Cochran-Armitage Trend Test (d)	P=0.181		
Fisher Exact Test (d)		P=0.122	P=0.306

TABLE C3. ANALYSES OF PRIMARY TUMORS IN MALE MICE IN THE TWO-YEAR FEED STUDY OF *N*-PHENYL-2-NAPHTHYLAMINE (Continued)

	Control	2,500 ppm	5,000 ppm
Liver: Hepatocellular Carcinoma			
Overall Rates (a)	6/47 (13%)	5/50 (10%)	9/47 (19%)
Adjusted Rates (b)	15.7%	13.9%	28.1%
Terminal Rates (c)	2/33 (6%)	5/36 (14%)	6/28 (21%)
Week of First Observation	87	104	86
Life Table Tests (d)	P=0.158	P=0.450N	P=0.210
Incidental Tumor Tests (d)	P=0.234	P=0.588N	P=0.314
Cochran-Armitage Trend Test (d)	P=0.228		
Fisher Exact Test (d)		P=0.456N	P=0.287
Liver: Hepatocellular Adenoma or Carcinoma			
Overall Rates (a)	11/47 (23%)	16/50 (32%)	17/47 (36%)
Adjusted Rates (b)	25.2%	41.9%	52.6%
Terminal Rates (c)	7/33 (21%)	14/36 (39%)	12/28 (46%)
Week of First Observation	87	77	86
Life Table Tests (d)	P=0.046	P=0.262	P=0.064
Incidental Tumor Tests (d)	P=0.062	P=0.183	P=0.108
Cochran-Armitage Trend Test (d)	P=0.109		
Fisher Exact Test (d)		P=0.237	P=0.130
Adrenal Gland: Cortical Adenoma			
Overall Rates (a)	3/48 (6%)	(f) 0/12 (0%)	1/46 (2%)
Adjusted Rates (b)	9.1%		3.6%
Terminal Rates (c)	3/33 (9%)		1/28 (4%)
Week of First Observation	104		104
Life Table Test (d)			P=0.266N
Incidental Tumor Test (d)			P=0.266N
Fisher Exact Test (d)			P=0.325N
Adrenal Gland Capsule: Adenoma			
Overall Rates (a)	10/48 (21%)	(f) 0/12 (0%)	6/46 (13%)
Adjusted Rates (b)	30.3%		21.4%
Terminal Rates (c)	10/33 (30%)		6/28 (21%)
Week of First Observation	104		104
Life Table Test (d)			P=0.212N
Incidental Tumor Test (d)			P=0.212N
Fisher Exact Test (d)			P=0.233N
All Sites: Benign Tumors			
Overall Rates (a)	19/49 (39%)	20/50 (40%)	18/50 (36%)
Adjusted Rates (b)	52.9%	51.1%	57.6%
Terminal Rates (c)	17/33 (52%)	17/36 (47%)	15/28 (54%)
Week of First Observation	87	77	63
Life Table Test (d)	P=0.387	P=0.531N	P=0.450
Incidental Tumor Test (d)	P=0.467	P=0.548	P=0.496
Cochran-Armitage Trend Test (d)	P=0.428N		
Fisher Exact Test (d)		P=0.532	P=0.469N
All Sites: Malignant Tumors			
Overall Rates (a)	23/49 (47%)	14/50 (28%)	23/50 (46%)
Adjusted Rates (b)	53.1%	33.0%	59.0%
Terminal Rates (c)	13/33 (39%)	8/36 (22%)	12/28 (43%)
Week of First Observation	84	69	81
Life Table Test (d)	P=0.358	P=0.047N	P=0.395
Incidental Tumor Test (d)	P=0.530N	P=0.098N	P=0.591N
Cochran-Armitage Trend Test (d)	P=0.508N		
Fisher Exact Test (d)		P=0.041N	P=0.543N

TABLE C3. ANALYSES OF PRIMARY TUMORS IN MALE MICE IN THE TWO-YEAR FEED STUDY OF *N*-PHENYL-2-NAPHTHYLAMINE (Continued)

	Control	2,500 ppm	5,000 ppm
All Sites: All Tumors			
Overall Rates (a)	34/49 (69%)	39/50 (80%)	32/50 (64%)
Adjusted Rates (b)	77.0%	89.6%	80.0%
Terminal Rates (c)	33/33 (70%)	22/36 (64%)	20/28 (71%)
Week of First Observation	64	69	63
Life Table Test (d)	P=0.277	P=0.176N	P=0.394
Incidental Tumor Test (d)	P=0.548N	P=0.410N	P=0.654N
Cochran-Armitage Trend Test (d)	P=0.326N		
Fisher Exact Test (d)		P=0.232N	P=0.361N

(a) Number of tumor-bearing animals/number of animals examined at the site
(b) Kaplan-Meier estimated tumor incidences at the end of the study after adjusting for intercurrent mortality
(c) Observed tumor incidence at terminal kill
(d) Beneath the control incidence are the P values associated with the trend test. Beneath the dosed group incidence are the P values corresponding to pairwise comparisons between that dosed group and the controls. The life table analysis regards tumors in animals dying prior to terminal kill as being (directly or indirectly) the cause of death. The incidental tumor test regards these lesions as nonfatal. The Cochran-Armitage and Fisher exact tests compare directly the overall incidence rates. A negative trend or lower incidence in a dosed group is indicated by (N).
(e) Only 16 spleens were examined.
(f) Incomplete sampling of tissues

TABLE C4a. HISTORICAL INCIDENCE OF HEPATOCELLULAR TUMORS IN MALE B6C3F$_1$ MICE RECEIVING NO TREATMENT (a)

Study	Incidence in Controls		
	Adenoma	Carcinoma	Adenoma or Carcinoma
Historical Incidence at Battelle Columbus Laboratories			
Chlorobenzene	7/50	14/50	19/50
C.I. Acid Orange 10	1/50	14/50	15/50
FD&C Yellow No. 6	1/50	12/50	13/50
C.I. Acid Red 14	8/48	10/48	15/48
C.I. Disperse Yellow 3	7/50	14/50	20/50
D&C Red No. 9	4/50	4/50	8/50
C.I. Solvent Yellow 14	5/49	10/49	15/49
l-Ascorbic acid	6/50	10/50	16/50
TOTAL	37/397 (9.3%)	89/397 (22.4%)	121/397 (30.5%)
SD (b)	4.94%	6.83%	7.37%
Range (c)			
High	7/50	14/50	20/50
Low	1/50	4/50	8/50
Overall Historical Incidence			
TOTAL	228/2,084 (10.9%)	424/2,084 (20.3%)	627/2,084 (30.1%)
SD (b)	7.29%	6.86%	7.78%
Range (c)			
High	(d) 22/50	16/50	(e) 29/50
Low	0/49	4/50	8/50

(a) Data as of August 30, 1985, for studies of at least 104 weeks
(b) Standard deviation
(c) Range and SD are presented for groups of 35 or more animals.
(d) Second highest: 11/50
(e) Second highest: 20/50

TABLE C4b. HISTORICAL INCIDENCE OF INTEGUMENTARY SYSTEM TUMORS IN MALE B6C3F$_1$ MICE RECEIVING NO TREATMENT (a)

Study	Incidence in Controls		
	Fibroma (b)	Fibrosarcoma (c)	Fibroma or Fibrosarcoma (b,c)
Historical Incidence at Battelle Columbus Laboratories			
Chlorobenzene	1/50	1/50	2/50
C.I. Acid Orange 10	0/50	6/50	6/50
FD&C Yellow No. 6	0/50	4/50	4/50
C.I. Acid Red 14	0/49	4/49	4/49
C.I. Disperse Yellow 3	0/50	0/50	0/50
D&C Red No. 9	0/50	2/50	2/50
C.I. Solvent Yellow 14	0/49	0/49	0/49
l-Ascorbic acid	0/50	1/50	1/50
TOTAL	1/298 (0.3%)	18/298 (4.5%)	19/298 (4.3%)
SD (d)	0.71%	4.39%	4.39%
Range (e)			
High	1/50	6/50	6/50
Low	0/50	0/50	0/50
Overall Historical Incidence			
TOTAL	36/2,091 (1.7%)	125/2,091 (6.0%)	156/2,091 (7.5%)
SD (d)	2.78%	6.48%	7.68%
Range (e)			
High	6/50	15/50	19/50
Low	0/50	0/50	0/50

(a) Data as of August 30, 1995, for studies of at least 104 weeks
(b) Includes neurofibromas
(c) Includes sarcomas, NOS, and neurofibrosarcomas
(d) Standard deviation
(e) Range and SD are presented for groups of 35 or more animals.

TABLE C5. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE MICE IN THE TWO-YEAR FEED STUDY OF *N*-PHENYL-2-NAPHTHYLAMINE

	Untreated Control	Low Dose	High Dose
ANIMALS INITIALLY IN STUDY	50	50	50
ANIMALS MISSING	1		
ANIMALS NECROPSIED	49	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY	49	50	50
INTEGUMENTARY SYSTEM			
*Skin	(49)	(50)	(50)
Inflammation, acute			3 (6%)
Abscess, NOS	1 (2%)	1 (2%)	
Inflammation, chronic	1 (2%)	5 (10%)	2 (4%)
*Subcutaneous tissue	(49)	(50)	(50)
Inflammation, acute diffuse			1 (2%)
RESPIRATORY SYSTEM			
#Lung	(49)	(50)	(46)
Hemorrhage			1 (2%)
Inflammation, suppurative	2 (4%)		1 (2%)
Hemosiderosis		1 (2%)	
Alveolar macrophages	6 (12%)	2 (4%)	3 (7%)
Hyperplasia, alveolar epithelium	4 (8%)	6 (12%)	1 (2%)
HEMATOPOIETIC SYSTEM			
*Multiple organs	(49)	(50)	(50)
Hyperplasia, lymphoid	1 (2%)		
#Bone marrow	(48)	(11)	(49)
Hyperplasia, granulocytic	1 (2%)	4 (36%)	8 (16%)
#Spleen	(45)	(16)	(48)
Depletion, lymphoid	1 (2%)	1 (6%)	5 (10%)
Hyperplasia, lymphoid			1 (2%)
Hematopoiesis	7 (16%)	5 (31%)	9 (19%)
#Mandibular lymph node	(44)	(22)	(44)
Inflammation, suppurative			1 (2%)
#Lumbar lymph node	(44)	(22)	(44)
Edema, NOS	1 (2%)		
Hyperplasia, lymphoid		1 (5%)	
#Mesenteric lymph node	(44)	(22)	(44)
Angiectasis	6 (14%)	9 (41%)	11 (25%)
#Renal lymph node	(44)	(22)	(44)
Hyperplasia, lymphoid		1 (5%)	
#Inguinal lymph node	(44)	(22)	(44)
Inflammation, acute/chronic	1 (2%)		
Hyperplasia, diffuse	2 (5%)		1 (2%)
#Thymic lymph node	(44)	(22)	(44)
Hematopoiesis	1 (2%)		
#Liver	(47)	(50)	(47)
Erythrophagocytosis		1 (2%)	
#Thymus	(37)	(9)	(24)
Necrosis, diffuse		4 (44%)	
Depletion, lymphoid	4 (11%)	1 (11%)	1 (4%)
CIRCULATORY SYSTEM			
#Lung	(49)	(50)	(46)
Thrombosis, NOS	1 (2%)		
#Heart	(49)	(11)	(47)
Periarteritis	1 (2%)		

TABLE C5. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE MICE IN THE TWO-YEAR FEED STUDY OF *N*-PHENYL-2-NAPHTHYLAMINE (Continued)

	Untreated Control	Low Dose	High Dose
CIRCULATORY SYSTEM (Continued)			
#Heart/atrium	(49)	(11)	(47)
Inflammation, suppurative			1 (2%)
#Myocardium	(49)	(11)	(47)
Degeneration, NOS	1 (2%)		5 (11%)
Necrosis, focal	1 (2%)		
#Urinary bladder/serosa	(43)	(12)	(48)
Periarteritis	1 (2%)		
DIGESTIVE SYSTEM			
*Periodontal tissues	(49)	(50)	(50)
Inflammation, acute/chronic	2 (4%)		4 (8%)
#Liver	(47)	(50)	(47)
Inflammation, acute/chronic		3 (6%)	
Necrosis, focal	5 (11%)	3 (6%)	7 (15%)
Cytoplasmic vacuolization	2 (4%)		1 (2%)
Basophilic cyto change	2 (4%)	2 (4%)	1 (2%)
Clear cell change		1 (2%)	
#Pancreas	(45)	(11)	(46)
Dilatation/ducts	1 (2%)	1 (9%)	
#Pancreatic acinus	(45)	(11)	(46)
Cytoplasmic vacuolization	1 (2%)		
Atrophy, focal	3 (7%)	2 (18%)	1 (2%)
Hyperplasia, focal			1 (2%)
#Peripancreatic tissue	(45)	(11)	(46)
Inflammation, acute focal	1 (2%)		
*Jejunal lumen	(49)	(50)	(50)
Hemorrhage			1 (2%)
*Rectal lumen	(49)	(50)	(50)
Hemorrhage			1 (2%)
#Glandular stomach	(43)	(10)	(46)
Inflammation, acute	1 (2%)		2 (4%)
#Duodenum	(41)	(9)	(43)
Ulcer, chronic			1 (2%)
#Jejunal mucosa	(41)	(9)	(43)
Amyloid, NOS	1 (2%)		
#Ileum	(41)	(9)	(43)
Mineralization			1 (2%)
Inflammation, acute/chronic			1 (2%)
*Rectum	(49)	(50)	(50)
Inflammation, acute hemorrhagic			1 (2%)
*Rectal mucosa	(49)	(50)	(50)
Necrosis, NOS	5 (10%)		1 (2%)
URINARY SYSTEM			
#Kidney	(49)	(50)	(47)
Cyst, NOS		1 (2%)	
Pyelonephritis, acute	2 (4%)	1 (2%)	4 (9%)
Pyelonephritis, chronic		1 (2%)	2 (4%)
Nephropathy	30 (61%)	32 (64%)	31 (66%)
Infarct, NOS	2 (4%)		3 (6%)
Hyperplasia, tubular cell		1 (2%)	2 (4%)
Metaplasia, osseous	1 (2%)		
#Kidney/tubule	(49)	(50)	(47)
Mineralization	4 (8%)		
Dilatation, NOS	2 (4%)	1 (2%)	2 (4%)
Necrosis, focal	1 (2%)		2 (4%)
Atrophy, focal		1 (2%)	

TABLE C5. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE MICE IN THE TWO-YEAR FEED STUDY OF *N*-PHENYL-2-NAPHTHYLAMINE (Continued)

	Untreated Control	Low Dose	High Dose
URINARY SYSTEM (Continued)			
#Urinary bladder	(43)	(12)	(48)
Calculus, gross observation only	1 (2%)		
Inflammation, acute necrotizing			4 (8%)
Inflammation, acute/chronic		1 (8%)	2 (4%)
*Urethra	(49)	(50)	(50)
Dilatation, NOS	1 (2%)		
Obstruction, NOS		1 (2%)	2 (4%)
Inflammation, suppurative			1 (2%)
ENDOCRINE SYSTEM			
#Adrenal/capsule	(48)	(12)	(46)
Hyperplasia, focal	38 (79%)		43 (93%)
#Adrenal cortex	(48)	(12)	(46)
Cytoplasmic vacuolization			1 (2%)
Hypertrophy, focal	3 (6%)		8 (17%)
Hyperplasia, focal	11 (23%)		6 (13%)
#Adrenal medulla	(48)	(12)	(46)
Hyperplasia, focal	1 (2%)		
#Thyroid	(48)	(11)	(47)
Inflammation, chronic	1 (2%)		
Hyperplasia, follicular cell	2 (4%)		
REPRODUCTIVE SYSTEM			
*Penis	(49)	(50)	(50)
Inflammation, suppurative	1 (2%)		2 (4%)
*Prepuce	(49)	(50)	(50)
Inflammation, acute necrotizing	3 (6%)	1 (2%)	3 (6%)
*Preputial gland	(49)	(50)	(50)
Dilatation/ducts			1 (2%)
Inflammation, acute/chronic	10 (20%)	5 (10%)	9 (18%)
#Prostate	(49)	(11)	(49)
Retention of content	1 (2%)		
Inflammation, suppurative	2 (4%)	2 (18%)	4 (8%)
*Seminal vesicle	(49)	(50)	(50)
Retention of content	6 (12%)	2 (4%)	4 (8%)
Inflammation, suppurative	1 (2%)		
Inflammation, chronic focal	2 (4%)	2 (4%)	
#Testis	(49)	(11)	(50)
Necrosis, diffuse		1 (9%)	
#Testis/tubule	(49)	(11)	(50)
Degeneration, NOS	4 (8%)		5 (10%)
NERVOUS SYSTEM			
None			
SPECIAL SENSE ORGANS			
None			
MUSCULOSKELETAL SYSTEM			
*Tarsal joint	(49)	(50)	(50)
Hyperostosis	25 (51%)	22 (44%)	14 (28%)
Metaplasia, osseous	25 (51%)	22 (44%)	14 (28%)

TABLE C8. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE MICE IN THE TWO-YEAR FEED STUDY OF *N*-PHENYL-2-NAPHTHYLAMINE (Continued)

	Untreated Control	Low Dose	High Dose
BODY CAVITIES			
*Peritoneum	(49)	(50)	(50)
Cyst, NOS		1 (2%)	
Inflammation, suppurative			1 (2%)
ALL OTHER SYSTEMS			
*Multiple organs	(49)	(50)	(50)
Inflammation, suppurative	1 (2%)		
Adipose tissue			
Inflammation, chronic diffuse	1	1	
SPECIAL MORPHOLOGY SUMMARY			
No lesion reported		1	2
Animal missing/no necropsy	1		

* Number of animals receiving complete necropsy examinations; all gross lesions including masses examined microscopically.
# Number of animals examined microscopically at this site

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APPENDIX D

SUMMARY OF LESIONS IN FEMALE MICE IN THE TWO-YEAR FEED STUDY OF *N*-PHENYL-2-NAPHTHYLAMINE

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TABLE D1. SUMMARY OF THE INCIDENCE OF NEOPLASMS IN FEMALE MICE IN THE TWO-YEAR FEED STUDY OF *N*-PHENYL-2-NAPHTHYLAMINE

	Untreated Control	Low Dose	High Dose
ANIMALS INITIALLY IN STUDY	50	50	50
ANIMALS NECROPSIED	50	50	48
ANIMALS EXAMINED HISTOPATHOLOGICALLY	50	50	48
INTEGUMENTARY SYSTEM			
*Skin	(50)	(50)	(48)
Basal cell carcinoma		1 (2%)	
*Subcutaneous tissue	(50)	(50)	(48)
Sarcoma, NOS	1 (2%)		1 (2%)
Osteosarcoma			1 (2%)
RESPIRATORY SYSTEM			
#Lung	(50)	(9)	(47)
Alveolar/bronchiolar adenoma	3 (6%)	1 (11%)	3 (6%)
Alveolar/bronchiolar carcinoma	2 (4%)		
Follicular cell carcinoma, metastatic	1 (2%)		
C-cell carcinoma, metastatic	1 (2%)		
Sarcoma, NOS, metastatic	1 (2%)	1 (11%)	1 (2%)
Osteosarcoma, metastatic			1 (2%)
HEMATOPOIETIC SYSTEM			
*Multiple organs	(50)	(50)	(48)
Malignant lymphoma, undiffer type	6 (12%)	1 (2%)	
Malignant lymphoma, lymphocytic type	6 (12%)	4 (8%)	8 (17%)
Malignant lymphoma, histiocytic type	4 (8%)	2 (4%)	5 (10%)
Malignant lymphoma, mixed type	4 (8%)		1 (2%)
#Spleen	(49)	(20)	(46)
Malignant lymphoma, lymphocytic type	2 (4%)		1 (2%)
Malignant lymphoma, mixed type	1 (2%)	1 (4%)	
#Mandibular lymph node	(48)	(14)	(45)
Sarcoma, NOS, metastatic			1 (2%)
#Mesenteric lymph node	(48)	(14)	(45)
Mast cell tumor	1 (2%)		
#Peyer's patch	(47)	(9)	(42)
Malignant lymphoma, lymphocytic type	1 (2%)		
#Kidney	(50)	(50)	(47)
Malignant lymphoma, lymphocytic type		1 (2%)	
#Uterus	(48)	(24)	(47)
Malignant lymphoma, histiocytic type			1 (2%)
CIRCULATORY SYSTEM			
*Subcutaneous tissue	(50)	(50)	(48)
Hemangiosarcoma		1 (2%)	
#Ovary	(48)	(22)	(45)
Hemangioma		1 (5%)	
DIGESTIVE SYSTEM			
*Tongue	(50)	(50)	(48)
Squamous cell carcinoma	1 (2%)		
#Liver	(50)	(14)	(48)
Hepatocellular adenoma	3 (6%)	2 (14%)	4 (8%)
Hepatocellular carcinoma	1 (2%)	1 (7%)	3 (6%)
#Forestomach	(49)	(8)	(47)
Squamous cell papilloma	1 (2%)		
#Cecum	(47)	(8)	(43)
Leiomyosarcoma		1 (13%)	

TABLE D1. SUMMARY OF THE INCIDENCE OF NEOPLASMS IN FEMALE MICE IN THE TWO-YEAR FEED STUDY OF *N*-PHENYL-2-NAPHTHYLAMINE (Continued)

	Untreated Control	Low Dose	High Dose
URINARY SYSTEM			
#Kidney	(50)	(50)	(47)
Tubular cell adenoma			1 (2%)
Tubular cell adenocarcinoma			1 (2%)
ENDOCRINE SYSTEM			
#Pituitary intermedia	(44)	(11)	(44)
Adenoma, NOS	1 (2%)	1 (9%)	
#Anterior pituitary	(44)	(11)	(44)
Carcinoma, NOS			1 (2%)
Adenoma, NOS	7 (16%)	4 (36%)	2 (5%)
#Adrenal	(48)	(7)	(48)
Cortical adenoma			1 (2%)
#Adrenal capsule	(48)	(7)	(48)
Adenoma, NOS	3 (6%)		1 (2%)
#Adrenal medulla	(48)	(7)	(48)
Pheochromocytoma			1 (2%)
#Thyroid	(50)	(5)	(47)
Follicular cell adenoma	1 (2%)		
Follicular cell carcinoma	1 (2%)		
C-cell carcinoma	1 (2%)		
REPRODUCTIVE SYSTEM			
*Mammary gland	(50)	(50)	(48)
Adenocarcinoma, NOS	2 (4%)		2 (4%)
#Uterus	(48)	(34)	(47)
Sarcoma, NOS		1 (3%)	1 (2%)
Leiomyoma	1 (2%)		
Endometrial stromal polyp		2 (6%)	
#Ovary	(48)	(22)	(45)
Luteoma	1 (2%)		
Granulosa cell tumor			1 (2%)
Teratoma, NOS		1 (5%)	
NERVOUS SYSTEM			
None			
SPECIAL SENSE ORGANS			
*Harderian gland	(50)	(50)	(48)
Adenoma, NOS	2 (4%)	1 (2%)	2 (4%)
Adenocarcinoma, NOS	1 (2%)		
MUSCULOSKELETAL SYSTEM			
None			
BODY CAVITIES			
None			
ALL OTHER SYSTEMS			
None			

TABLE D1. SUMMARY OF THE INCIDENCE OF NEOPLASMS IN FEMALE MICE IN THE TWO-YEAR FEED STUDY OF *N*-PHENYL-2-NAPHTHYLAMINE (Continued)

	Untreated Control	Low Dose	High Dose
ANIMAL DISPOSITION SUMMARY			
Animals initially in study	50	50	50
Natural death	10	13	11
Moribund sacrifice	4	8	6
Terminal sacrifice	36	29	34
TUMOR SUMMARY			
Total animals with primary tumors**	42	23	33
Total primary tumors	58	27	42
Total animals with benign tumors	18	10	15
Total benign tumors	22	12	15
Total animals with malignant tumors	31	13	26
Total malignant tumors	34	14	26
Total animals with secondary tumors##	3	1	2
Total secondary tumors	3	1	3
Total animals with tumors uncertain--benign or malignant	1	1	1
Total uncertain tumors	1	1	1

* Number of animals receiving complete necropsy examinations; all gross lesions including masses examined microscopically.
** Primary tumors: all tumors except secondary tumors
# Number of animals examined microscopically at this site
Secondary tumors: metastatic tumors or tumors invasive into an adjacent organ

TABLE D8. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF FEMALE MICE IN THE TWO-YEAR FEED STUDY OF *N*-PHENYL-2-NAPHTHYLAMINE: UNTREATED CONTROL

ANIMAL NUMBER	0 2 2	0 2 3	0 3 0	0 3 2	0 3 3	0 4 0	0 4 1	0 4 2	0 4 3	0 4 4	0 5 0	0 5 1	0 5 2	0 5 3	0 5 4	0 6 0	0 6 1	0 6 2	0 6 3	0 6 4	0 7 0	0 7 1	0 7 2	0 7 3	0 7 4	0 8 0	0 8 1	0 8 2	0 8 3	0 8 4	0 9 0	0 9 1	0 9 2	0 9 3	0 9 4	0 10 0	0 10 1	0 10 2	0 10 3	0 10 4
WEEKS ON STUDY	0 0 1	0 0 1	0 0 1	0 0 1	0 0 1	0 0 1	0 0 1	0 0 1	0 0 1	0 0 1	0 0 1	0 0 1	0 0 1	0 0 1	0 0 1	0 0 1	0 0 1	0 0 1	0 0 1	0 0 1	0 0 1	0 0 1	0 0 1	0 0 1	0 0 1	0 0 1	0 0 1	0 0 1	0 0 1	0 0 1	0 0 1	0 0 1	0 0 1	0 0 1	0 0 1	0 0 1	0 0 1	0 0 1	0 0 1	0 0 1
INTEGUMENTARY SYSTEM																																							
Subcutaneous tissue	+	M	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Sarcoma, NOS					X																																		
RESPIRATORY SYSTEM																																							
Lung and bronchi	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma						X	X																												X				
Alveolar/bronchiolar carcinoma																																							
Follicular cell carcinoma, metastatic					X																																		
C-cell carcinoma, metastatic																																							
Sarcoma, NOS, metastatic					+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea																																							
HEMATOPOIETIC SYSTEM																																							
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spleen	+	+	+	+	+	+	+	+	+	+	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Malignant lymphoma, lymphocytic type																																							
Malignant lymphoma, mixed type																																							
Lymph nodes	+	+	+	+	+	+	+	+	+	–	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Mast cell tumor																X																							
Thymus	+	–	+	+	–	–	+	–	–	–	–	+	+	+	+	+	+	+	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
CIRCULATORY SYSTEM																																							
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
DIGESTIVE SYSTEM																																							
Oral cavity	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M
Squamous cell carcinoma		X																																					
Salivary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatocellular adenoma																																							
Hepatocellular carcinoma	X																																						
Bile duct	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Gallbladder & common bile duct	M	M	+	+	M	+	X	+	+	M	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pancreas	+	+	+	+	+	+	+	+	+	–	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach	+	+	+	+	+	+	+	+	+	+	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Squamous cell papilloma																																							
Small intestine	+	–	+	+	+	+	+	+	+	–	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Malignant lymphoma, lymphocytic type																																				X			
Large intestine	+	–	+	+	+	+	+	+	+	–	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
URINARY SYSTEM																																							
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Urinary bladder	+	–	+	+	+	+	+	+	+	–	–	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
ENDOCRINE SYSTEM																																							
Pituitary	–	+	+	–	+	+	+	+	+	+	+	–	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma, NOS																X	X	X						X	X															
Adrenal	+	–	+	+	+	+	+	+	+	+	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma, NOS		–																																					
Thyroid	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Follicular cell adenoma																																							
Follicular cell carcinoma																																				X			
C-cell carcinoma																																							
Parathyroid	+	+	–	+	+	–	–	+	+	+	+	+	+	+	+	+	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
REPRODUCTIVE SYSTEM																																							
Mammary gland	M	M	+	M	+	M	+	+	+	M	M	M	+	M	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenocarcinoma, NOS																																							
Uterus	+	+	+	+	+	+	+	+	+	+	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leiomyoma																																							
Ovary	+	+	+	+	+	+	+	+	+	+	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Luteoma																								X																
NERVOUS SYSTEM																																							
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
SPECIAL SENSE ORGANS																																							
Harderian gland	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M
Adenoma, NOS																X																								
Adenocarcinoma, NOS																																							
ALL OTHER SYSTEMS																																							
Multiple organs, NOS	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M
Malignant lymphoma, undifferentiated type			X							X																													
Malignant lymphoma, lymphocytic type				X																X	X					X														
Malignant lymphoma, histiocytic type						X						X	X																											
Malignant lymphoma, mixed type																	X																							

TABLE D2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF FEMALE MICE: UNTREATED CONTROL (Continued)

ANIMAL NUMBER	0 0 1	0 0 2	0 0 3	0 0 4	0 0 5	0 0 6	0 0 7	0 0 8	0 0 9	0 1 0	0 1 1	0 1 2	0 1 3	0 1 4	0 1 5	0 1 6	0 1 7	0 1 8	0 1 9	0 2 0	0 2 1	0 2 2	0 2 3	0 2 4	0 2 5	TOTAL: TISSUES TUMORS
WEEKS ON STUDY	0 4	0 4	0 4	0 4	0 4	0 4	0 4	0 4	0 4	0 4	0 4	0 4	0 4	0 4	0 4	0 4	0 4	0 4	0 4	0 4	0 4	0 4	0 4	0 4	0 4	
INTEGUMENTARY SYSTEM																										
Subcutaneous tissue	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	*50
Sarcoma, NOS																										1
RESPIRATORY SYSTEM																										
Lungs and bronchi	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar adenoma										X						X									X	3
Alveolar/bronchiolar carcinoma																										2
Follicular cell carcinoma, metastatic																										1
C-cell carcinoma, metastatic																										1
Sarcoma, NOS, metastatic																				X						1
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
HEMATOPOIETIC SYSTEM																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Malignant lymphoma, lymphocytic type					X																X					2
Malignant lymphoma, mixed type																				X						1
Lymph nodes	+	+	+	+	+	+	+	+	+	○	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Mast cell tumor																										1
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	+	41
CIRCULATORY SYSTEM																										
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
DIGESTIVE SYSTEM																										
Oral cavity	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	*50
Squamous cell carcinoma																										1
Salivary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hepatocellular adenoma											X	X										X				3
Hepatocellular carcinoma																										1
Bile duct	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Gallbladder & common bile duct	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	*50
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Squamous cell papilloma																X										1
Small intestine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Malignant lymphoma, lymphocytic type																										1
Large intestine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
URINARY SYSTEM																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Urinary bladder	+	+	+	+	+	+	+	+	+	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	45
ENDOCRINE SYSTEM																										
Pituitary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	+	44
Adenoma, NOS					X					X										X						5
Adrenal	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Adenoma, NOS					X														X				X			3
Thyroid	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Follicular cell adenoma																				X						1
Follicular cell carcinoma																										1
C-cell carcinoma																			X							1
Parathyroid	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
REPRODUCTIVE SYSTEM																										
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	N	+	+	+	+	+	+	+	+	+	+	N	+	*50
Adenocarcinoma, NOS																			X	X						3
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Leiomyoma												X														1
Ovary	+	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Luteoma																										1
NERVOUS SYSTEM																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
SPECIAL SENSE ORGANS																										
Harderian gland	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	*50
Adenoma, NOS	X	X																								2
Adenocarcinoma, NOS																										1
ALL OTHER SYSTEMS																										
Multiple organs, NOS	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	*50
Malignant lymphoma, undiff type						X									X			X								6
Malignant lymphoma, lymphocytic type							X		X																	6
Malignant lymphoma, histiocytic type										X																4
Malignant lymphoma, mixed type															X		X						X			4

* Animals necropsied

TABLE D8. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF FEMALE MICE IN THE TWO-YEAR FEED STUDY OF *N*-PHENYL-2-NAPHTHYLAMINE: LOW DOSE

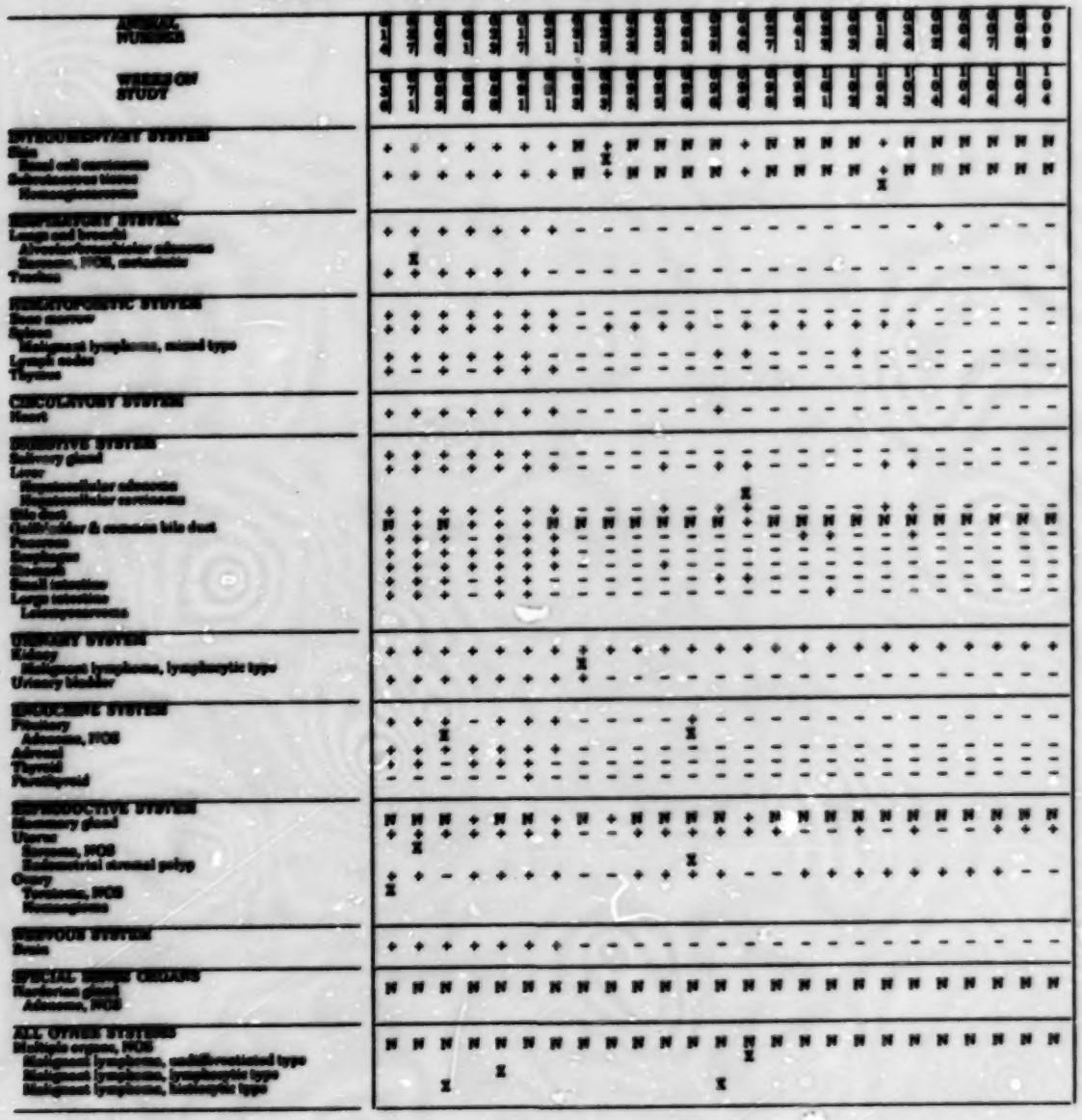

TABLE D8. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF FEMALE MICE: LOW DOSE (Continued)

ANIMAL NUMBER	0 1 1	0 1 2	0 1 3	0 1 4	0 1 5	0 1 6	0 1 7	0 1 8	0 1 9	0 2 0	0 2 1	0 2 2	0 2 3	0 2 4	0 2 5	0 2 6	0 2 7	0 2 8	0 2 9	0 3 0	0 3 1	0 3 2	0 3 3	0 3 4	0 3 5	0 3 6	0 3 7	0 3 8	0 3 9	0 4 0	TOTAL: TISSUES TUMORS
WEEKS ON STUDY	0 4 1	0 4 1	0 4 1	0 4 1	0 4 1	0 4 1	0 4 1	0 4 1	0 4 1	0 4 1	0 4 1	0 4 1	0 4 1	0 4 1	0 4 1	0 4 1	0 4 1	0 4 1	0 4 1	0 4 1	0 4 1	0 4 1	0 4 1	0 4 1	0 4 1	0 4 1	0 4 1	0 4 1	0 4 1	0 4 1	
INTEGUMENTARY SYSTEM																															
Skin	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	*50
Basal cell carcinoma																															1
Subcutaneous tissue	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	*50
Hemangiosarcoma																															1
RESPIRATORY SYSTEM																															
Lungs and bronchi	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	9
Alveolar/bronchiolar adenoma														X																	1
Sarcoma, NOS, metastatic																															1
Trachea	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	6
HEMATOPOIETIC SYSTEM																															
Bone marrow	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	7
Spleen	-	-	+	-	+	-	-	+	-	+	-	-	-	-	-	-	-	-	+	-	-	+	-	-	-	-	-	+	-	-	25
Malignant lymphoma, mixed type																						X									1
Lymph nodes	-	-	-	-	+	-	-	+	-	+	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	+	-	-	14
Thymus	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	6
CIRCULATORY SYSTEM																															
Heart	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	8
DIGESTIVE SYSTEM																															
Salivary gland	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	7
Liver	-	-	+	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	14
Hepatocellular adenoma			X											X																	2
Hepatocellular carcinoma																															1
Bile duct	-	-	+	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1
Gallbladder & common bile duct	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	14
Pancreas	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	*50
Esophagus	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	9
Stomach	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	7
Small intestine	-	-	-	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	8
Large intestine	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	9
Leiomyosarcoma						X																									1
URINARY SYSTEM																															
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Malignant lymphoma, lymphocytic type																															1
Urinary bladder	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
ENDOCRINE SYSTEM																															
Pituitary	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-	+	-	-	-	-	+	-	-	-	-	-	-	-	11
Adenoma, NOS											X							X					X								5
Adrenal	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	7
Thyroid	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	5
Parathyroid	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1
REPRODUCTIVE SYSTEM																															
Mammary gland	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	*50
Uterus	+	-	-	+	+	+	+	-	+	-	-	+	+	+	+	+	+	-	-	-	+	+	+	+	-	+	-	-	-	+	34
Sarcoma, NOS																X															1
Endometrial stromal polyp																															3
Ovary	+	+	+	-	-	+	+	+	+	+	-	-	+	+	-	-	-	-	-	+	+	-	-	-	+	+	-	-	-	-	22
Teratoma, NOS																															1
Hemangioma														X																	1
NERVOUS SYSTEM																															
Brain	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	7
SPECIAL SENSE ORGANS																															
Harderian gland	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	*50
Adenoma, NOS																															1
ALL OTHER SYSTEMS																															
Multiple organs, NOS	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	*50
Malignant lymphoma, undiffer type																															1
Malignant lymphoma, lymphocytic type					X			X		X																					4
Malignant lymphoma, histiocytic type																															2

* Animals necropsied

TABLE D8. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF FEMALE MICE IN THE TWO-YEAR FEED STUDY OF *N*-PHENYL-2-NAPHTHYLAMINE: HIGH DOSE

ANIMAL NUMBER	3 1 5	4 4 6	4 4 8	3 2 6	3 3 5	4 1 2	3 2 8	4 1 6	3 3 2	3 3 6	4 1 8	3 4 0	4 1 4	3 4 4	4 2 0	4 2 2	4 2 4	4 2 6	4 2 8	4 3 0	4 3 2	4 3 4	4 3 6	4 3 8	4 4 0
WEEKS ON STUDY	0 3 5	1 3 6	1 3 7	1 3 8	1 3 8	1 3 8	1 3 8	1 3 8	1 3 8	1 3 8	1 3 8	1 3 8	1 3 8	1 3 8	1 4 0	1 4 0	1 4 0	1 4 0	1 4 0	1 4 0	1 4 0	1 4 0	1 4 0	1 4 0	1 4 4
INTEGUMENTARY SYSTEM																									
Subcutaneous tissue	+	B	B	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Sarcoma, NOS								X																	
Osteosarcoma										X															
RESPIRATORY SYSTEM																									
Lungs and bronchi	+	B	B	+	+	+	+	+	+	+	+	–	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma							X											X							
Sarcoma, NOS, metastatic								X																	
Osteosarcoma, metastatic										X															
Trachea	+	B	B	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
HEMATOPOIETIC SYSTEM																									
Bone marrow	+	B	B	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spleen	–	B	B	+	+	+	+	+	+	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Malignant lymphoma, lymphocytic type																									X
Lymph nodes	+	B	B	+	+	+	+	+	+	–	+	+	–	–	+	+	+	+	+	+	+	+	+	+	+
Sarcoma, NOS, metastatic								X																	
Thymus	–	B	B	–	+	+	+	–	–	–	+	–	+	–	+	+	+	+	+	+	+	+	+	+	+
CIRCULATORY SYSTEM																									
Heart	+	B	B	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
DIGESTIVE SYSTEM																									
Salivary gland	+	B	B	+	+	+	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Liver	+	B	B	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatocellular adenoma				X														X							
Hepatocellular carcinoma														X								X			
Bile duct	+	B	B	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Gallbladder & common bile duct	M	B	B	M	M	+	+	+	M	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pancreas	–	B	B	+	+	+	+	+	–	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Esophagus	+	B	B	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach	–	B	B	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Small intestine	–	B	B	–	+	+	+	+	–	–	+	+	–	+	+	+	+	+	+	+	+	+	+	+	+
Large intestine	–	B	B	+	+	+	+	+	–	–	+	+	–	+	+	+	+	+	+	+	+	+	+	+	+
URINARY SYSTEM																									
Kidney	–	B	B	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Tubular cell adenoma																				X					
Tubular cell adenocarcinoma																									
Urinary bladder	+	B	B	+	+	+	+	+	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
ENDOCRINE SYSTEM																									
Pituitary	+	B	B	+	+	+	+	+	–	–	+	+	–	–	+	+	+	+	+	+	+	+	+	+	+
Carcinoma, NOS																									
Adenoma, NOS																									
Adrenal	+	B	B	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma, NOS																									
Cortical adenoma																									
Pheochromocytoma																									X
Thyroid	+	B	B	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Parathyroid	–	B	B	–	–	+	+	–	+	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
REPRODUCTIVE SYSTEM																									
Mammary gland	+	B	B	N	N	+	+	N	+	N	N	N	+	N	+	+	+	+	+	+	+	+	+	+	+
Adenocarcinoma, NOS																									
Uterus	+	B	B	+	+	+	+	+	+	+	+	+	–	+	+	+	+	+	+	+	+	+	+	+	+
Sarcoma, NOS																									
Malignant lymphoma, histocytic type																									
Ovary	+	B	B	+	+	+	+	+	+	+	+	+	–	+	+	+	+	+	+	+	+	+	+	+	+
Granulosa cell tumor																									
NERVOUS SYSTEM																									
Brain	+	B	B	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
SPECIAL SENSE ORGANS																									
Harderian gland	N	B	B	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Adenoma, NOS																									
ALL OTHER SYSTEMS																									
Multiple organs, NOS	M	B	B	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Malignant lymphoma, lymphocytic type							X						X					X							
Malignant lymphoma, histocytic type					X												X			X				
Malignant lymphoma, mixed type									X																

+: Tissue examined microscopically
–: Required tissue not examined microscopically
X: Tumor incidence
M: Necropsy, no autolysis, no microscopic examination
S: Animal missing

.: No tissue information submitted
C: Necropsy, no histology due to protocol
A: Autolysis
M: Animal missing
B: No necropsy performed

TABLE D2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF FEMALE MICE: HIGH DOSE (Continued)

ANIMAL NUMBER	0 1 2	0 1 3	0 1 4	0 1 5	0 1 8	0 1 9	0 2 0	0 2 1	0 2 2	0 2 3	0 2 4	0 2 5	0 2 6	0 2 7	0 2 8	0 2 9	0 3 0	0 3 1	0 3 2	0 3 3	0 3 4	0 3 5	0 3 7	0 3 8	0 3 9	0 4 0	0 4 2	0 4 4	0 4 5	0 5 0	TOTAL: TISSUES TUMORS
WEEKS ON STUDY	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	
INTEGUMENTARY SYSTEM																															
Subcutaneous tissue	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	*48
Sarcoma, NOS																															1
Osteosarcoma																															1
RESPIRATORY SYSTEM																															
Lungs and bronchi	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Alveolar/bronchiolar adenoma				X																											3
Sarcoma, NOS, metastatic																															1
Osteosarcoma, metastatic																															1
Trachea	+	+	+	+	+	+	+	+	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
HEMATOPOIETIC SYSTEM																															
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
Malignant lymphoma, lymphocytic type																															1
Lymph nodes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	45
Sarcoma, NOS, metastatic																															1
Thymus	+	+	+	+	–	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	+	+	–	+	+	+	+	37
CIRCULATORY SYSTEM																															
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
DIGESTIVE SYSTEM																															
Salivary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Hepatocellular adenoma			X																			X									4
Hepatocellular carcinoma																										X					3
Bile duct	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Gallbladder & common bile duct	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	*48
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	45
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Small intestine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Large intestine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	+	+	+	42
	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	+	+	+	43
URINARY SYSTEM																															
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Tubular cell adenoma																						X									1
Tubular cell adenocarcinoma																															1
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
ENDOCRINE SYSTEM																															
Pituitary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	44
Carcinoma, NOS																					X										1
Adenoma, NOS														X															X		2
Adrenal	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Adenoma, NOS																														X	1
Cortical adenoma																										X					1
Pheochromocytoma																															1
Thyroid	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Parathyroid	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	42
REPRODUCTIVE SYSTEM																															
Mammary gland	+	+	+	+	+	+	N	+	N	+	N	+	+	+	+	+	+	N	+	N	+	+	+	N	N	+	+				*48
Adenocarcinoma, NOS				X									X																		2
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+				47
Sarcoma, NOS																						X									1
Malignant lymphoma, histocytic type			X																												1
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	–	+	+	+	+	+	+	+	–	+	+	+	+	+	+				45
Granulosa cell tumor														X																	1
NERVOUS SYSTEM																															
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+				48
SPECIAL SENSE ORGANS																															
Harderian gland	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N				*48
Adenoma, NOS					X							X																			2
ALL OTHER SYSTEMS																															
Multiple organs, NOS	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N				*48
Malignant lymphoma, lymphocytic type					X		X	X												X						X					8
Malignant lymphoma, histiocytic type	X																														5
Malignant lymphoma, mixed type																															1

* Animals necropsied

TABLE D3. ANALYSIS OF PRIMARY TUMORS IN FEMALE MICE IN THE TWO-YEAR FEED STUDY OF *N*-PHENYL-2-NAPHTHYLAMINE

	Control	2,500 ppm	5,000 ppm
Lung: Alveolar/Bronchiolar Adenoma			
Overall Rates (a)	3/50 (6%)	(b) 1/9 (11%)	3/47 (6%)
Adjusted Rates (c)	8.3%		7.8%
Terminal Rates (d)	3/36 (8%)		2/35 (6%)
Week of First Observation	104		92
Life Table Test (e)			P=0.655
Incidental Tumor Test (e)			P=0.609
Fisher Exact Test (e)			P=0.631
Lung: Alveolar/Bronchiolar Adenoma or Carcinoma			
Overall Rates (a)	5/50 (10%)	(b) 1/9 (11%)	3/47 (6%)
Adjusted Rates (c)	12.4%		7.8%
Terminal Rates (d)	3/36 (8%)		2/35 (6%)
Week of First Observation	89		92
Life Table Test (e)			P=0.366N
Incidental Tumor Test (e)			P=0.483N
Fisher Exact Test (e)			P=0.393N
Hematopoietic System: Malignant Lymphoma, Undifferentiated Type			
Overall Rates (a)	6/50 (12%)	(b,f) 1/50 (2%)	0/48 (0%)
Adjusted Rates (c)	15.0%		0.0%
Terminal Rates (d)	4/36 (11%)		0/35 (0%)
Week of First Observation	86		
Life Table Test (e)			P=0.021N
Incidental Tumor Test (e)			P=0.024N
Fisher Exact Test (e)			P=0.015N
Hematopoietic System: Malignant Lymphoma, Lymphocytic Type			
Overall Rates (a)	9/50 (18%)	(b,f) 5/50 (10%)	9/48 (19%)
Adjusted Rates (c)	22.6%		23.7%
Terminal Rates (d)	6/36 (17%)		7/35 (20%)
Week of First Observation	86		92
Life Table Test (e)			P=0.573
Incidental Tumor Test (e)			P=0.548
Fisher Exact Test (e)			P=0.565
Hematopoietic System: Malignant Lymphoma, Histiocytic Type			
Overall Rates (a)	4/50 (8%)	(b,f) 2/50 (4%)	6/48 (13%)
Adjusted Rates (c)	9.6%		16.1%
Terminal Rates (d)	1/36 (3%)		5/35 (14%)
Week of First Observation	89		88
Life Table Test (e)			P=0.354
Incidental Tumor Test (e)			P=0.310
Fisher Exact Test (e)			P=0.344
Hematopoietic System: Malignant Lymphoma, Mixed Type			
Overall Rates (a)	5/50 (10%)	(b,f) 1/50 (2%)	1/48 (2%)
Adjusted Rates (c)	13.9%		2.3%
Terminal Rates (d)	5/36 (14%)		0/35 (0%)
Week of First Observation	104		94
Life Table Test (e)			P=0.110N
Incidental Tumor Test (e)			P=0.105N
Fisher Exact Test (e)			P=0.112N
Hematopoietic System: Lymphoma, All Malignant			
Overall Rates (a)	24/50 (48%)	(b,f) 9/50 (18%)	16/48 (33%)
Adjusted Rates (c)	54.2%		40.1%
Terminal Rates (d)	16/36 (44%)		12/35 (34%)
Week of First Observation	86		88
Life Table Test (e)			P=0.118N
Incidental Tumor Test (e)			P=0.122N
Fisher Exact Test (e)			P=0.102N

TABLE D3. ANALYSIS OF PRIMARY TUMORS IN FEMALE MICE IN THE TWO-YEAR FEED STUDY OF *N*-PHENYL-2-NAPHTHYLAMINE (Continued)

	Control	2,500 ppm	5,000 ppm
Liver: Hepatocellular Adenoma			
Overall Rates (a)	3/50 (6%)	(b) 2/14 (14%)	4/48 (8%)
Adjusted Rates (c)	8.3%		10.5%
Terminal Rates (d)	3/36 (8%)		3/35 (9%)
Week of First Observation	104		82
Life Table Test (e)			P=0.484
Incidental Tumor Test (e)			P=0.444
Fisher Exact Test (e)			P=0.477
Liver: Hepatocellular Carcinoma			
Overall Rates (a)	1/50 (2%)	(b) 1/14 (7%)	3/48 (6%)
Adjusted Rates (c)	2.0%		8.3%
Terminal Rates (d)	0/36 (0%)		2/35 (6%)
Week of First Observation	80		102
Life Table Test (e)			P=0.293
Incidental Tumor Test (e)			P=0.266
Fisher Exact Test (e)			P=0.293
Liver: Hepatocellular Adenoma or Carcinoma			
Overall Rates (a)	4/50 (8%)	(b) 3/14 (21%)	7/48 (15%)
Adjusted Rates (c)	10.2%		18.4%
Terminal Rates (d)	3/36 (8%)		5/35 (14%)
Week of First Observation	80		82
Life Table Test (e)			P=0.246
Incidental Tumor Test (e)			P=0.205
Fisher Exact Test (e)			P=0.239
Pituitary Gland: Adenoma			
Overall Rates (a)	7/44 (16%)	(b) 4/11 (36%)	2/44 (5%)
Adjusted Rates (c)	20.6%		5.7%
Terminal Rates (d)	7/34 (21%)		2/35 (6%)
Week of First Observation	104		104
Life Table Test (e)			P=0.071N
Incidental Tumor Test (e)			P=0.071N
Fisher Exact Test (e)			P=0.079N
Adrenal Gland Capsule: Adenoma			
Overall Rates (a)	3/48 (6%)	(b) 0/7 (0%)	1/48 (2%)
Adjusted Rates (c)	8.3%		2.9%
Terminal Rates (d)	3/36 (8%)		1/35(3%)
Week of First Observation	104		104
Life Table Test (e)			P=0.315N
Incidental Tumor Test (e)			P=0.315N
Fisher Exact Test (e)			P=0.309N
All Sites: Benign Tumors			
Overall Rates (a)	18/50 (36%)	10/50 (20%)	15/48 (31%)
Adjusted Rates (c)	48.6%	30.0%	39.8%
Terminal Rates (d)	17/36 (47%)	8/30 (27%)	13/35 (37%)
Week of First Observation	102	83	82
Life Table Test (e)	P=0.323N	P=0.144N	P=0.366N
Incidental Tumor Test (e)	P=0.356N	P=0.114N	P=0.404N
Cochran-Armitage Trend Test (e)	P=0.335N		
Fisher Exact Test (e)		P=0.069N	P=0.389N

TABLE D3. ANALYSIS OF PRIMARY TUMORS IN FEMALE MICE IN THE TWO-YEAR FEED STUDY OF *N*-PHENYL-2-NAPHTHYLAMINE (Continued)

	Control	2,500 ppm	5,000 ppm
All Sites: Malignant Tumors			
Overall Rates (a)	31/50 (62%)	13/50 (26%)	26/48 (54%)
Adjusted Rates (c)	64.3%	31.4%	61.4%
Terminal Rates (d)	19/36 (53%)	5/30 (17%)	19/35 (54%)
Week of First Observation	80	71	86
Life Table Test (e)	P=0.265N	P=0.008N	P=0.301N
Incidental Tumor Test (e)	P=0.301N	P<0.001N	P=0.364N
Cochran-Armitage Trend Test (e)	P=0.236N		
Fisher Exact Test (e)		P<0.001N	P=0.281N
All Sites: All Tumors			
Overall Rates (a)	42/50 (84%)	23/50 (46%)	33/48 (69%)
Adjusted Rates (c)	87.4%	55.5%	76.4%
Terminal Rates (d)	30/36 (83%)	13/30 (43%)	25/35 (71%)
Week of First Observation	80	30	82
Life Table Test (e)	P=0.106N	P=0.013N	P=0.113N
Incidental Tumor Test (e)	P=0.078N	P<0.001N	P=0.106N
Cochran-Armitage Trend Test (e)	P=0.063N		
Fisher Exact Test (e)		P<0.001N	P=0.061N

(a) Number of tumor-bearing animals/number of animals examined at the site
(b) Incomplete sampling of tissues
(c) Kaplan-Meier estimated tumor incidences at the end of the study after adjusting for intercurrent mortality
(d) Observed tumor incidence at terminal kill
(e) Beneath the control incidences are the P values associated with the trend test. Beneath the high dose group incidence are the P values corresponding to pairwise comparisons between that dosed group and the controls. The life table analysis regards tumors in animals dying prior to terminal kill as being (directly or indirectly) the cause of death. The incidental tumor test regards these lesions as nonfatal. The Cochran-Armitage and Fisher exact tests compare directly the overall incidence rates. A negative trend or lower incidence in a dosed group is indicated by (N).
(f) Only 14 livers, 26 spleens, 14 lymph nodes, 6 thymuses, and 7 bone marrow samples were examined microscopically.

TABLE D4. HISTORICAL INCIDENCE OF KIDNEY TUMORS IN FEMALE B6C3F$_1$ MICE RECEIVING NO TREATMENT (a)

Historical Incidence at Battelle Columbus Laboratories			
	No. Examined	No. of Tumors	Diagnosis
	394	0	
Overall Historical Incidence			
		1	Tubular cell adenoma
		1	Tubular cell adenocarcinoma
TOTAL	2,079	(b) 1 (0.05%)	

(a) Data as of August 30, 1985, for studies of at least 104 weeks
(b) Both tumors were observed in the same animal in the oxytetracycline hydrochloride study.

TABLE D6. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE MICE IN THE TWO-YEAR FEED STUDY OF *N*-PHENYL-2-NAPHTHYLAMINE

	Untreated Control	Low Dose	High Dose
ANIMALS INITIALLY IN STUDY	50	50	50
ANIMALS NECROPSIED	50	50	48
ANIMALS EXAMINED HISTOPATHOLOGICALLY	50	50	48
INTEGUMENTARY SYSTEM			
*Subcutaneous tissue	(50)	(50)	(48)
Inflammation, suppurative	1 (2%)		
RESPIRATORY SYSTEM			
#Lung	(50)	(9)	(47)
Hemorrhage			1 (2%)
Inflammation, suppurative	3 (6%)		1 (2%)
Infarct, NOS			1 (2%)
Alveolar macrophages	3 (6%)		1 (2%)
Hyperplasia, alveolar epithelium			1 (2%)
HEMATOPOIETIC SYSTEM			
*Multiple organs	(50)	(50)	(48)
Hyperplasia, lymphoid	1 (2%)		
#Bone marrow	(50)	(7)	(48)
Necrosis, NOS			1 (2%)
Hyperplasia, granulocytic	5 (10%)	2 (29%)	9 (19%)
#Spleen	(48)	(36)	(46)
Amyloid, NOS			1 (2%)
Hyperplasia, lymphoid	2 (4%)	1 (4%)	1 (2%)
Hematopoiesis	6 (12%)	17 (68%)	8 (17%)
#Mandibular lymph node	(48)	(14)	(48)
Hemorrhage			1 (2%)
Necrosis, focal			1 (2%)
#Bronchial lymph node	(48)	(14)	(48)
Edema, NOS			1 (2%)
#Lumbar lymph node	(48)	(14)	(45)
Dilatation/sinus	1 (2%)		
Angiectasis			1 (2%)
#Mesenteric lymph node	(48)	(14)	(46)
Angiectasis	2 (4%)	1 (7%)	3 (7%)
#Renal lymph node	(48)	(14)	(46)
Edema, NOS		1 (7%)	
Inflammation, suppurative		1 (7%)	
Angiectasis			1 (2%)
*Cranial and facial bones	(50)	(50)	(48)
Myelofibrosis	31 (62%)		35 (73%)
*Femur	(50)	(50)	(48)
Myelofibrosis	24 (48%)		33 (69%)
#Liver	(50)	(14)	(48)
Hematopoiesis	1 (2%)	3 (21%)	5 (10%)
#Adrenal cortex	(48)	(7)	(48)
Hematopoiesis			1 (2%)
#Thymus	(41)	(8)	(37)
Depletion, lymphoid	1 (2%)		
CIRCULATORY SYSTEM			
#Brain/meninges	(50)	(7)	(48)
Periarteritis			1 (2%)
#Lung	(50)	(9)	(47)
Thrombosis, NOS	1 (2%)		

TABLE D5. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE MICE IN THE TWO-YEAR FEED STUDY OF *N*-PHENYL-2-NAPHTHYLAMINE (Continued)

	Untreated Control	Low Dose	High Dose
CIRCULATORY SYSTEM (Continued)			
#Heart	(50)	(5)	(48)
Thrombosis, NOS	1 (2%)		
Inflammation, acute focal	1 (2%)		1 (2%)
Periarteritis			4 (8%)
#Myocardium	(50)	(8)	(48)
Degeneration, NOS	1 (2%)		
*Uterine artery	(50)	(50)	(48)
Inflammation, fibrinoid	1 (2%)		
*Adrenal artery	(50)	(50)	(48)
Inflammation, fibrinoid	1 (2%)		
#Liver	(50)	(14)	(48)
Thrombosis, NOS	1 (2%)		
#Pancreas	(47)	(9)	(45)
Periarteritis			1 (2%)
#Kidney	(50)	(50)	(47)
Embolus, septic	1 (2%)		
#Ovary	(48)	(32)	(45)
Thrombosis, NOS	1 (2%)		
DIGESTIVE SYSTEM			
#Liver	(50)	(14)	(48)
Inflammation, acute/chronic	2 (4%)		2 (4%)
Necrosis, focal	15 (30%)	6 (43%)	15 (30%)
Cytoplasmic vacuolization	2 (4%)	1 (7%)	
Basophilic cyto change	1 (2%)		2 (4%)
#Pancreas	(47)	(9)	(45)
Dilatation/ducts	1 (2%)	1 (11%)	1 (2%)
Inflammation, chronic	1 (2%)		
Inflammation, granulomatous focal	1 (2%)		
#Pancreatic acinus	(47)	(9)	(45)
Cytoplasmic vacuolization	1 (2%)		
Atrophy, focal	4 (9%)	1 (11%)	3 (7%)
#Periesophageal tissue	(50)	(7)	(48)
Inflammation, suppurative	1 (2%)		
#Glandular stomach	(48)	(8)	(47)
Inflammation, acute	1 (2%)		1 (2%)
#Forestomach	(49)	(5)	(47)
Ulcer, chronic			1 (2%)
Hyperplasia, epithelial	1 (2%)		
#Ileal mucosa	(47)	(9)	(42)
Inflammation, acute necrotizing		1 (11%)	
Amyloid, NOS	1 (2%)		
#Colon	(47)	(8)	(43)
Parasitism	3 (6%)		
*Rectal mucosa	(50)	(50)	(48)
Necrosis, NOS	1 (2%)		
URINARY SYSTEM			
#Kidney	(50)	(50)	(47)
Hydronephrosis		1 (2%)	
Pyelonephritis, acute	1 (2%)		1 (2%)
Pyelonephritis, chronic	1 (2%)		2 (4%)
Nephropathy	18 (36%)	16 (32%)	32 (68%)
Infarct, NOS	5 (10%)	1 (2%)	1 (2%)
Amyloidosis	2 (4%)		1 (2%)
Nuclear enlargement			17 (36%)
Hyperplasia, tubular cell			2 (4%)
Metaplasia, carcinoma	1 (2%)		

TABLE D6. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE MICE IN THE TWO-YEAR FEED STUDY OF *N*-PHENYL-2-NAPHTHYLAMINE (Continued)

	Untreated Control	Low Dose	High Dose
URINARY SYSTEM (Continued)			
#Kidney/tubule	(50)	(50)	(47)
Mineralization			1 (2%)
Dilatation, NOS	1 (2%)		1 (2%)
Necrosis, focal	3 (6%)		
Inclusion, nuclear		1 (2%)	
Atrophy, focal	1 (2%)		
#Urinary bladder	(45)	(8)	(47)
Inflammation, acute/chronic			1 (2%)
ENDOCRINE SYSTEM			
#Anterior pituitary	(44)	(11)	(44)
Hemorrhage			1 (2%)
Cytologic alteration, NOS	1 (2%)		
Hyperplasia, focal	9 (20%)	1 (9%)	12 (27%)
Angiectasis	1 (2%)	1 (9%)	2 (5%)
#Adrenal/capsule	(48)	(7)	(48)
Hyperplasia, focal	46 (96%)		46 (96%)
Hyperplasia, diffuse	2 (4%)		
#Adrenal cortex	(48)	(7)	(48)
Degeneration, lipoid	2 (4%)		
Hyperplasia, focal	1 (2%)		
#Adrenal medulla	(48)	(7)	(48)
Hyperplasia, focal	2 (4%)	1 (14%)	2 (4%)
#Thyroid	(50)	(8)	(47)
Inflammation, chronic			1 (2%)
Hyperplasia, follicular cell	4 (8%)		
REPRODUCTIVE SYSTEM			
*Mammary gland	(50)	(50)	(48)
Hyperplasia, cystic	3 (6%)		1 (2%)
#Uterus	(49)	(34)	(47)
Dilatation, NOS	3 (6%)		1 (2%)
Inflammation, suppurative	8 (16%)	8 (24%)	16 (34%)
Inflammation, granulomatous focal	1 (2%)		
Angiectasis		1 (3%)	1 (2%)
#Uterus/endometrium	(49)	(34)	(47)
Hyperplasia, cystic	32 (65%)	20 (59%)	23 (49%)
#Fallopian tube	(49)	(34)	(47)
Inflammation, suppurative			2 (4%)
#Ovary/paraovarian	(48)	(32)	(45)
Inflammation, acute/chronic	1 (2%)	1 (3%)	
Inflammation, granulomatous focal	1 (2%)		
#Ovary	(48)	(32)	(45)
Cyst, NOS	14 (29%)	10 (31%)	20 (44%)
Multiple cysts	1 (2%)	9 (28%)	4 (9%)
Hemorrhagic cyst	1 (2%)		2 (4%)
Abscess, chronic	3 (6%)	13 (41%)	8 (18%)
Hyperplasia, epithelial			1 (2%)
NERVOUS SYSTEM			
#Brain	(50)	(7)	(48)
Infarct, NOS	1 (2%)	1 (14%)	1 (2%)
Atrophy, pressure	2 (4%)	1 (14%)	1 (2%)

TABLE D6. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE MICE IN THE TWO-YEAR FEED STUDY OF *N*-PHENYL-2-NAPHTHYLAMINE (Continued)

	Untreated Control	Low Dose	High Dose
SPECIAL SENSE ORGANS			
*Eye	(50)	(50)	(48)
Inflammation, suppurative			1 (2%)
*Eye/crystalline lens	(50)	(50)	(48)
Cataract	1 (2%)		
*Harderian gland	(50)	(50)	(48)
Hyperplasia, focal		1 (2%)	
MUSCULOSKELETAL SYSTEM			
*Tarsal joint	(50)	(50)	(48)
Hyperostosis			1 (2%)
Metaplasia, osseous			1 (2%)
BODY CAVITIES			
*Peritoneum	(50)	(50)	(48)
Inflammation, suppurative	3 (6%)	12 (24%)	6 (13%)
Necrosis, fat	3 (6%)	1 (2%)	
*Pleura	(50)	(50)	(48)
Inflammation, suppurative		1 (2%)	
ALL OTHER SYSTEMS			
*Multiple organs	(50)	(50)	(48)
Inflammation, suppurative	1 (2%)		
Inflammation, acute/chronic			1 (2%)
SPECIAL MORPHOLOGY SUMMARY			
No lesion reported		1	
No necropsy performed			2

* Number of animals receiving complete necropsy examinations; all gross lesions including masses examined microscopically.
# Number of animals examined microscopically at this site

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APPENDIX E

GENETIC TOXICOLOGY OF *N*-PHENYL-2-NAPHTHYLAMINE

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TABLE E1. MUTAGENICITY OF *N*-PHENYL-2-NAPHTHYLAMINE IN *SALMONELLA TYPHIMURIUM* (a)

Strain	Dose (µg/plate)	Revertants/plate (b)					
		−S9		+S9 (hamster)		+S9 (rat)	
		Trial 1	Trial 2	10% S9	30% S9	10% S9	30% S9
TA100	0	92 ± 3.8	143 ± 4.3	126 ± 16.4	122 ± 7.8	135 ± 5.0	136 ± 12.8
	0.1	105 ± 12.8	136 ± 7.1	--	--	--	--
	0.3	103 ± 6.1	129 ± 7.2	--	--	--	--
	1	99 ± 3.2	129 ± 10.7	--	--	--	--
	3	96 ± 6.2	135 ± 10.4	120 ± 9.6	128 ± 9.6	138 ± 10.3	166 ± 11.8
	6	--	(c) 88 ± 11.9	--	--	--	--
	10	(c) 0 ± 0.0	--	130 ± 17.5	147 ± 12.0	131 ± 15.6	159 ± 11.1
	33	--	--	122 ± 7.0	137 ± 4.8	125 ± 0.3	147 ± 3.5
	100	--	--	98 ± 12.5	125 ± 5.9	121 ± 4.1	144 ± 4.4
	166	--	--	--	126 ± 6.2	--	130 ± 10.4
	333	--	--	(c) 31 ± 2.5	--	(c) 98 ± 5.8	--
	Trial summary	Negative	Negative	Negative	Negative	Negative	Negative
	Positive control (d)	455 ± 47.7	549 ± 26.5	1,344 ± 103.2	538 ± 42.5	456 ± 10.2	303 ± 16.6
TA1535	0	27 ± 5.5	26 ± 1.2	7 ± 0.6	11 ± 0.7	11 ± 2.3	20 ± 2.3
	0.1	25 ± 3.5	29 ± 2.6	--	--	--	--
	0.3	25 ± 2.6	30 ± 3.5	--	--	--	--
	1	24 ± 3.3	34 ± 2.3	--	--	--	--
	3	22 ± 2.6	31 ± 1.2	10 ± 2.8	16 ± 1.3	7 ± 0.9	22 ± 1.5
	6	--	35 ± 1.8	--	--	--	--
	10	(c) 2 ± 0.9	--	16 ± 1.2	15 ± 1.3	9 ± 2.0	23 ± 2.3
	33	--	--	11 ± 1.2	15 ± 4.8	10 ± 1.9	16 ± 1.2
	100	--	--	14 ± 2.4	11 ± 1.9	12 ± 2.1	14 ± 1.7
	166	--	--	--	16 ± 1.9	--	13 ± 1.3
	333	--	--	(c) 4 ± 0.9	--	(c) 5 ± 2.0	--
	Trial summary	Negative	Negative	Negative	Negative	Negative	Negative
	Positive control (d)	332 ± 18.8	553 ± 19.8	448 ± 20.8	525 ± 8.8	158 ± 13.1	239 ± 7.2
TA97	0	102 ± 2.1	127 ± 3.5	129 ± 5.8	166 ± 13.7	162 ± 8.7	192 ± 2.4
	0.1	96 ± 0.7	144 ± 8.4	--	--	--	--
	0.3	124 ± 0.9	139 ± 7.0	--	--	--	--
	1	104 ± 2.7	141 ± 8.4	--	--	--	--
	3	97 ± 7.0	149 ± 4.7	155 ± 5.7	159 ± 9.2	144 ± 2.9	192 ± 7.1
	6	--	(c) 81 ± 4.7	--	--	--	--
	10	(c) 41 ± 7.8	--	158 ± 9.0	166 ± 9.8	149 ± 0.3	186 ± 10.0
	33	--	--	151 ± 1.8	161 ± 11.1	137 ± 1.2	192 ± 3.3
	100	--	--	129 ± 7.5	156 ± 11.2	113 ± 9.0	192 ± 6.6
	166	--	--	--	144 ± 13.5	--	183 ± 6.2
	333	--	--	(c) 65 ± 6.8	--	(c) 88 ± 13.3	--
	Trial summary	Negative	Negative	Negative	Negative	Negative	Negative
	Positive control (d)	1,229 ± 27.9	996 ± 57.7	1,519 ± 14.7	1,099 ± 12.9	958 ± 38.2	441 ± 11.1
TA98	0	26 ± 4.5	23 ± 2.9	41 ± 4.3	32 ± 4.1	35 ± 4.7	48 ± 3.2
	0.1	22 ± 2.8	20 ± 2.2	--	--	--	--
	0.3	26 ± 5.5	27 ± 0.6	--	--	--	--
	1	16 ± 3.6	20 ± 3.4	--	--	--	--
	3	15 ± 0.7	17 ± 0.7	40 ± 7.7	33 ± 2.4	40 ± 3.5	47 ± 3.9
	6	--	21 ± 2.0	--	--	--	--
	10	(c) 10 ± 1.0	--	37 ± 3.4	44 ± 7.0	38 ± 1.0	49 ± 3.0
	33	--	--	37 ± 0.9	40 ± 2.7	31 ± 3.8	50 ± 4.0
	100	--	--	30 ± 3.9	32 ± 6.1	26 ± 0.9	33 ± 1.8
	166	--	--	--	28 ± 1.9	--	42 ± 5.5
	333	--	--	(c) 10 ± 2.7	--	(c) 21 ± 2.4	--
	Trial summary	Negative	Negative	Negative	Negative	Negative	Negative
	Positive control (d)	1,437 ± 25.8	1,372 ± 46.2	1,367 ± 25.7	388 ± 30.7	297 ± 21.7	184 ± 10.2

TABLE E1. MUTAGENICITY OF *N*-PHENYL-2-NAPHTHYLAMINE IN *SALMONELLA TYPHIMURIUM* (Continued)

(a) Study performed at SRI International. The detailed protocol is presented in Haworth et al. (1983). Cells and study compound or solvent (dimethyl sulfoxide) were incubated in the absence of exogenous metabolic activation (−S9) or with Aroclor 1254-induced S9 from male Syrian hamster liver or male Sprague Dawley rat liver. High dose was limited by toxicity or solubility but did not exceed 10 mg/plate; 0 µg/plate dose is the solvent control.
(b) Revertants are presented as mean ± standard error from three plates.
(c) Slight toxicity
(d) Positive control; 2-aminoanthracene was used on all strains in the presence of S9. In the absence of metabolic activation, 4-nitro-*o*-phenylenediamine was used with TA98, sodium azide was used with TA100 and TA1535, and 9-aminoacridine was used with TA97.

TABLE E2. INDUCTION OF SISTER CHROMATID EXCHANGES IN CHINESE HAMSTER OVARY CELLS BY *N*-PHENYL-2-NAPHTHYLAMINE (a)

Compound	Dose (µg/ml)	Total Cells	No. of Chromosomes	No. of SCEs	SCEs/ Chromosome	SCEs/ Cell	Hours in BrdU	Relative SCEs/Cell (percent) (b)
–S9 (c)								
Trial No. 1–Summary: Negative								
Dimethyl sulfoxide		50	1,021	356	0.35	7.1	26.5	--
N-Phenyl-2-naphthylamine								
	1.14	50	1,041	402	0.39	8.0	26.5	112.7
	3.41	50	1,036	348	0.34	7.0	26.5	98.6
	11.40	50	1,041	378	0.36	7.6	26.5	107.0
	34.10	0	--	--	--	--	(d) 31.0	--
Mitomycin C								
	0.002	50	1,033	510	0.49	10.2	26.5	143.7
	0.010	10	209	201	0.96	20.1	26.5	283.1
+S9 (c)								
Trial No. 1–Summary: Weakly positive								
Dimethyl sulfoxide		50	1,044	435	0.42	8.7	26.0	--
N-Phenyl-2-naphthylamine								
	3.41	50	1,046	503	0.48	10.1	26.0	116.1
	11.40	50	1,046	522	0.50	10.4	26.0	119.5
	34.10	50	1,042	530	0.51	10.6	(d) 30.0	121.8
	114.0	0	--	--	--	--	(d) 30.0	--
Cyclophosphamide								
	0.50	50	1,046	531	0.51	10.6	26.0	121.8
	2.50	10	211	229	1.13	23.9	26.0	274.7
Trial No. 2–Summary: Questionable								
Dimethyl sulfoxide		50	1,038	515	0.50	10.3	26.0	--
N-Phenyl-2-naphthylamine								
	5	50	1,042	611	0.59	12.2	26.0	118.4
	10	50	1,045	588	0.56	11.8	26.0	114.6
	20	50	1,042	519	0.50	10.4	26.0	101.0
	30	50	1,047	585	0.56	11.7	(d) 30.0	113.6
	40	10	208	161	0.77	16.1	(d) 30.0	156.3
Cyclophosphamide								
	0.50	50	1,043	692	0.66	13.8	26.0	134.0
	2.50	10	208	261	1.25	26.1	26.0	253.4

(a) Study performed at Biological Systems Corporation. SCE = sister chromatid exchange; BrdU = bromodeoxyuridine. A detailed description of the SCE protocol is presented by Galloway et al. (1985). Briefly, Chinese hamster ovary cells were incubated with study compound or solvent (dimethyl sulfoxide) as described in (c) or (e) below and cultured for sufficient time to reach second metaphase division. Cells were then collected by mitotic shake-off, fixed, air-dried, and stained.
(b) SCEs/cell of culture exposed to study chemical relative to those of culture exposed to solvent
(c) In the absence of S9, Chinese hamster ovary cells were incubated with study compound or solvent for 2 hours at 37° C. Then BrdU was added, and incubation was continued for 26 hours. Cells were washed, fresh medium containing BrdU and colcemid was added, and incubation was continued for 2-3 hours.
(d) Because some chemicals induce a delay in the cell division cycle, harvest times are occasionally extended to maximize the proportion of second division cells available for analysis.
(e) In the presence of S9, cells were incubated with study compound or solvent for 2 hours at 37° C. Then cells were washed, and medium containing BrdU was added. Cells were incubated for a further 26 hours, with colcemid present for the final 2-3 hours. S9 was from the liver of Aroclor 1254-induced male Sprague Dawley rats.

TABLE E3. INDUCTION OF CHROMOSOMAL ABERRATIONS IN CHINESE HAMSTER OVARY CELLS BY *N*-PHENYL-2-NAPHTHYLAMINE (a)

		−S9 (b)					+S9 (c)		
Dose (µg/ml)	Total Cells	No. of Abe	Abe/ Cell	Percent Cells with Abe	Dose (µg/ml)	Total Cells	No. of Abe	Abe/ Cell	Percent Cells with Abe
Trial 1--Harvest time 10.5 h					Trial 2--Harvest time 12.0 h				
Dimethyl sulfoxide					Dimethyl sulfoxide				
	200	7	0.04	3		200	12	0.06	6
N-Phenyl-2-naphthylamine					*N*-Phenyl-2-naphthylamine				
2.97	200	2	0.01	1	2.97	200	12	0.06	6
9.90	200	4	0.02	2	9.90	200	6	0.03	3
29.70	200	2	0.01	1	29.70	200	36	0.18	8
49.50	0	--	--	--	49.50	0	--	--	--
Mitomycin C					Cyclophosphamide				
1	200	31	0.16	14	50	50	21	0.42	28
5	50	18	0.36	22	--	--	--	--	--

(a) Study performed at Biological Systems Corporation. Abe = aberrations. A detailed presentation of the technique for detecting chromosomal aberrations is presented by Galloway et al. (1985). Briefly, Chinese hamster ovary cells were incubated with study compound or solvent (dimethyl sulfoxide) as indicated in (b) or (c). Cells were arrested in first metaphase by addition of colcemid and harvested by mitotic shake-off, fixed, and stained in 6% Giemsa.
(b) In the absence of S9, Chinese hamster ovary cells were incubated with study compound or solvent (dimethyl sulfoxide) for 8-10 hours at 37° C. Cells were then washed, and fresh medium containing colcemid was added for an additional 2-3 hours followed by harvest.
(c) In the presence of S9, cells were incubated with study compound or solvent (dimethyl sulfoxide) for 2 hours at 37° C. Cells were then washed, medium was added, and incubation was continued for 8-10 hours. Colcemid was added for the last 2-3 hours of incubation before harvest. S9 was from the liver of Aroclor 1254-induced male Sprague Dawley rats.

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APPENDIX F

SENTINEL ANIMAL PROGRAM

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APPENDIX F. SENTINEL ANIMAL PROGRAM

I. Methods

Rodents used in the Carcinogenesis Program of the National Toxicology Program are produced in optimally clean facilities to eliminate potential pathogens that may affect study results. The Sentinel Animal Program is part of the periodic monitoring of animal health that occurs during the toxicologic evaluation of chemical compounds. Under this program, the disease state of the rodents is monitored via serology on sera from extra (sentinel) animals in the study rooms. These animals are untreated, and these animals and the study animals are both subject to identical environmental conditions. The sentinel animals come from the same production source and weanling groups as the animals used for the studies of chemical compounds.

Fifteen B6C3F$_1$ mice and 15 F344/N rats of each sex are selected at the time of randomization and allocation of the animals to the various study groups. Five animals of each designated sentinel group are killed at 6, 12, and 18 months on study. Data from animals surviving 24 months are collected from 5/50 randomly selected control animals of each sex and species. The blood from each animal is collected and clotted, and the serum is separated. The serum is cooled on ice and shipped to Microbiological Associates' Comprehensive Animal Diagnostic Service for determination of the antibody titers. The following tests are performed:

	Hemagglutination Inhibition	Complement Fixation	ELISA
Mice	PVM (pneumonia virus of mice) Reo 3 (reovirus type 3) GDVII (Theiler's encephalomyelitis virus) Poly (polyoma virus) MVM (minute virus of mice) Ectro (infectious ectromelia) Sendai	M. Ad. (mouse adenovirus) LCM (lymphocytic choriomeningitis virus)	MHV (mouse hepatitis virus) *M. pul.* (*Mycoplasma pulmonis*) (24 mo)
Rats	PVM KRV (Kilham rat virus) H-1 (Toolan's H-1 virus) Sendai (6,12, 24 mo)	RCV (rat coronavirus) Sendai (18 mo)	

II. Results

Results are presented in Table F1.

TABLE F1. MURINE ANTIBODY DETERMINATIONS FOR RATS AND MICE IN THE TWO-YEAR FEED STUDIES OF *N*-PHENYL-2-NAPHTHYLAMINE (a)

Interval (months)	No. of Animals	Positive Serologic Reaction for
RATS		
6	--	None positive
12	--	None positive
18	--	None positive
24	--	None positive
MICE		
6	--	None positive
12	5/9	MVM
	2/10	MHV
18	4/10	Reo 3
24	10/10	MHV

(a) Blood samples were taken from sentinel animals at 6, 12, and 18 months after the start of dosing and from the control animals just before they were killed; samples were sent to Microbiological Associates (Bethesda, MD) for the Animal Disease Screening Program.

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APPENDIX G

FEED AND COMPOUND CONSUMPTION BY RATS AND MICE IN THE TWO-YEAR FEED STUDIES OF *N*-PHENYL-2-NAPHTHYLAMINE

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TABLE G1. FEED AND COMPOUND CONSUMPTION BY MALE RATS IN THE TWO-YEAR FEED STUDY OF *N*-PHENYL-2-NAPHTHYLAMINE

	Control		1,000 ppm				3,000 ppm			
Week	Grams Feed/ Day (a)	Body Weight (grams)	Grams Feed/ Day (a)	Body Weight (grams)	Low/ Control (b)	Dose/ Day (c)	Grams Feed/ Day (a)	Body Weight (grams)	High/ Control (b)	Dose/ Day (c)
1	15	152	13	178	0.9	183	13	180	0.9	361
5	23	267	13	288	0.6	128	16	244	0.7	328
9	18	312	18	297	1.0	152	17	283	0.9	330
12	16	342	15	323	0.9	116	18	301	1.1	299
16	13	371	13	348	1.0	93	12	335	0.9	179
21	16	392	15	377	0.9	99	15	335	0.9	224
25	20	414	18	394	0.9	114	19	371	1.0	256
30	23	436	23	416	1.0	139	23	389	1.0	296
34	16	451	15	425	0.9	98	15	404	0.9	186
37	18	447	17	431	0.9	99	17	411	0.9	207
42	17	468	16	444	0.9	90	17	417	1.0	204
46	14	468	13	448	0.9	73	13	422	0.9	154
50	17	482	16	460	0.9	87	16	432	0.9	185
54	18	475	18	452	1.0	100	18	427	1.0	211
60	16	477	16	476	1.0	84	16	429	1.0	186
64	16	477	16	451	1.0	89	16	434	1.0	189
68	17	489	16	467	0.9	85	17	430	1.0	198
72	16	487	16	444	1.0	90	16	421	0.9	178
77	17	486	15	447	0.9	84	15	422	0.9	178
81	17	479	15	441	0.9	85	16	414	0.9	193
86	17	482	17	433	1.0	98	17	407	1.0	209
91	16	478	17	437	1.1	97	18	409	1.1	220
95	17	483	16	432	0.9	92	18	402	1.1	224
98	16	468	16	425	1.0	94	19	398	1.2	245
Mean	17.0	430	16.0	404	0.9	103	16.5	379	1.0	225
SD (d)	2.3		2.1		0.1	25	2.3		0.1	54
CV (e)	13.5		13.1		11.1	24.3	13.9		10.0	24.0

(a) Grams of feed removed from feeder per animal per day. Not corrected for scatter.
(b) Grams of feed per day for the dosed group divided by that for the controls
(c) Estimated milligrams of *N*-phenyl-2-naphthylamine consumed per day per kilogram of body weight
(d) Standard deviation
(e) Coefficient of variation = (standard deviation/mean) × 100

TABLE G2. FEED AND COMPOUND CONSUMPTION BY FEMALE RATS IN THE TWO-YEAR FEED STUDY OF *N*-PHENYL-2-NAPHTHYLAMINE

	Control		2,500 ppm				5,000 ppm			
Week	Grams Feed/ Day (a)	Body Weight (grams)	Grams Feed/ Day (a)	Body Weight (grams)	Low/ Control (b)	Dose/ Day (c)	Grams Feed/ Day (a)	Body Weight (grams)	High/ Control (b)	Dose/ Day (c)
1	15	140	10	135	0.7	185	12	134	0.8	448
5	11	177	9	167	0.8	135	9	161	0.8	280
9	13	198	12	185	0.9	162	11	174	0.8	316
12	10	204	9	188	0.9	120	9	180	0.9	250
16	7	214	6	196	0.9	77	5	184	0.7	136
21	11	221	10	200	0.9	125	9	187	0.8	241
25	14	229	11	209	0.8	132	11	195	0.8	282
30	17	238	15	215	0.9	174	14	201	0.8	348
34	11	250	10	222	0.9	113	9	204	0.8	221
37	12	250	12	222	1.0	135	12	207	1.0	290
42	13	256	11	227	0.8	121	12	212	0.9	283
46	8	263	7	231	0.9	76	6	213	0.8	141
50	13	269	11	236	0.8	117	12	216	0.9	278
54	13	271	12	233	0.9	129	11	214	0.8	257
60	12	294	10	247	0.8	101	10	220	0.8	227
64	12	302	11	246	0.9	112	11	220	0.9	250
68	13	311	12	259	0.9	116	12	224	0.9	268
72	12	321	9	262	0.8	86	10	225	0.8	222
77	11	329	10	269	0.9	93	10	228	0.9	219
81	12	329	11	269	0.9	102	11	227	0.9	242
85	13	332	12	271	0.9	111	11	229	0.8	240
91	13	347	12	287	0.9	105	13	237	1.0	274
95	13	355	11	294	0.8	94	13	240	1.0	271
98	12	353	12	291	1.0	103	13	239	1.1	272
Mean	12.1	269	10.6	232	0.9	118	10.7	207	0.9	261
SD (d)	2.0		1.8		0.1	28	2.1		0.1	61
CV (e)	16.5		17.0		11.1	23.7	19.6		11.1	23.4

(a) Grams of feed removed from feeder per animal per day. Not corrected for scatter.
(b) Grams of feed per day for the dosed group divided by that for the controls
(c) Estimated milligrams of *N*-phenyl-2-naphthylamine consumed per day per kilogram of body weight
(d) Standard deviation
(e) Coefficient of variation = (standard deviation/mean) × 100

TABLE G5. FEED AND COMPOUND CONSUMPTION BY MALE MICE IN THE TWO-YEAR FEED STUDY OF *N*-PHENYL-2-NAPHTHYLAMINE

Week	Control: Grams Feed/ Day (a)	Control: Body Weight (grams)	2,500 ppm: Grams Feed/ Day (a)	2,500 ppm: Body Weight (grams)	2,500 ppm: Low/ Control (b)	2,500 ppm: Dose/ Day (c)	5,000 ppm: Grams Feed/ Day (a)	5,000 ppm: Body Weight (grams)	5,000 ppm: High/ Control (b)	5,000 ppm: Dose/ Day (c)
4	5	26.8	6	26.8	1.2	560	5	26.1	1.0	958
8	5	29.6	5	30.1	1.0	415	5	29.3	1.0	853
12	5	30.6	5	30.9	1.0	406	5	29.2	1.0	856
16	5	32.5	5	32.7	1.0	382	5	31.9	1.0	784
20	10	34.3	11	35.3	1.1	779	10	33.5	1.0	1,493
26	8	34.7	8	34.7	1.0	576	8	33.6	1.0	1,190
22	5	35.5	5	35.2	1.0	355	5	33.3	1.0	751
26	5	36.4	6	36.1	1.2	416	6	34.5	1.2	870
41	6	37.4	7	36.9	1.2	474	7	34.7	1.2	1,009
45	6	37.5	7	37.6	1.2	465	6	36.4	1.0	847
49	6	38.4	7	37.8	1.2	463	7	35.5	1.2	986
53	5	38.0	7	38.2	1.4	459	7	35.2	1.4	994
58	6	38.7	7	39.1	1.2	448	7	35.9	1.2	975
62	6	39.5	6	39.4	1.0	381	6	36.3	1.0	826
66	5	39.4	7	39.4	1.4	444	8	37.0	1.6	1,081
70	6	38.2	7	38.8	1.2	451	6	35.0	1.0	857
74	6	39.0	7	38.2	1.2	457	7	35.9	1.2	975
80	6	39.0	8	37.6	1.3	532	8	36.1	1.3	1,108
84	6	39.1	6	38.0	1.0	395	6	36.6	1.0	820
88	6	39.4	6	38.5	1.0	390	6	35.9	1.0	836
93	12	38.8	11	37.9	0.9	726	12	35.4	1.0	1,695
97	7	39.3	7	38.6	1.0	453	8	35.5	1.1	1,127
101	6	38.2	6	37.9	1.0	396	7	34.9	1.2	1,003
Mean	6.2	36.5	6.8	36.3	1.1	470	6.8	34.2	1.1	995
SD (d)	1.7		1.6		0.1	105	1.7		0.2	224
CV (e)	27.4		23.5		9.1	22.3	25.0		18.2	22.5

(a) Grams of feed removed from feeder per animal per day. Not corrected for scatter.
(b) Grams of feed per day for the dosed group divided by that for the controls
(c) Estimated milligrams of *N*-phenyl-2-naphthylamine consumed per day per kilogram of body weight
(d) Standard deviation
(e) Coefficient of variation = (standard deviation/mean) × 100

TABLE G4. FEED AND COMPOUND CONSUMPTION BY FEMALE MICE IN THE TWO-YEAR FEED STUDY OF *N*-PHENYL-2-NAPHTHYLAMINE

	Control		3,000 ppm				6,000 ppm			
Week	Grams Feed/ Day (a)	Body Weight (grams)	Grams Feed/ Day (a)	Body Weight (grams)	Low/ Control (b)	Dose/ Day (c)	Grams Feed/ Day (a)	Body Weight (grams)	High/ Control (b)	Dose/ Day (c)
4	5	22.2	5	21.6	1.0	579	5	21.2	1.0	1,179
8	4	23.4	5	23.1	1.3	641	5	23.0	1.3	1,087
12	4	24.5	5	23.6	1.2	530	3	22.5	0.8	687
16	3	25.7	3	25.5	1.0	294	3	24.9	1.0	602
20	9	27.6	10	26.7	1.1	936	8	25.6	0.9	1,563
26	5	28.2	7	27.8	1.4	639	6	26.0	1.2	1,154
32	3	28.7	3	27.8	1.0	270	2	26.3	0.7	380
36	4	30.7	5	29.7	1.3	421	4	27.5	1.0	727
41	5	31.3	5	30.1	1.0	415	4	27.1	0.8	738
45	5	32.1	5	31.5	1.0	397	5	28.4	1.0	880
49	6	33.2	6	31.5	1.0	476	5	28.5	0.8	877
53	5	33.1	5	32.0	1.0	391	5	27.7	1.0	903
58	5	35.2	5	34.1	1.0	367	4	29.4	0.8	680
62	5	36.2	5	35.1	1.0	356	4	30.0	0.8	667
66	4	36.6	6	36.1	1.5	416	6	29.4	1.5	1,020
70	5	37.3	5	35.2	1.0	355	5	30.0	1.0	833
74	4	36.6	5	34.0	1.3	368	4	28.4	1.0	704
80	5	38.1	5	35.9	1.0	348	5	28.3	1.0	883
84	4	39.7	4	37.1	1.0	270	4	31.2	1.0	641
88	5	39.1	5	36.9	1.0	339	4	30.2	0.8	662
93	9	40.4	7	38.2	0.8	458	9	30.8	1.0	1,461
97	5	41.1	5	39.1	1.0	328	7	32.1	1.4	1,090
101	5	39.8	5	38.0	1.0	329	6	30.6	1.2	980
Mean	5.0	33.1	5.2	31.7	1.1	427	4.9	27.8	1.0	886
SD (d)	1.5		1.4		0.2	146	1.6		0.2	280
CV (e)	30.0		26.4		18.2	34.2	32.7		20.0	31.6

(a) Grams of feed removed from feeder per animal per day. Not corrected for scatter.
(b) Grams of feed per day for the dosed group divided by that for the controls
(c) Estimated milligrams of *N*-phenyl-2-naphthylamine consumed per day per kilogram of body weight
(d) Standard deviation
(e) Coefficient of variation = standard deviation/mean × 100

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APPENDIX H

INGREDIENTS, NUTRIENT COMPOSITION, AND CONTAMINANT LEVELS IN NIH 07 RAT AND MOUSE RATION

Meal Diet: April 1981 to April 1983

(Manufactured by Zeigler Bros., Inc., Gardners, PA)

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TABLE H1. INGREDIENTS OF NIH 07 RAT AND MOUSE RATION (a)

Ingredient (b)	Percent by Weight
Ground #2 yellow shelled corn	24.50
Ground hard winter wheat	23.00
Soybean meal (49% protein)	12.00
Fish meal (60% protein)	10.00
Wheat middlings	10.00
Dried skim milk	5.00
Alfalfa meal (dehydrated, 17% protein)	4.00
Corn gluten meal (60% protein)	3.00
Soy oil	2.50
Brewer's dried yeast	2.00
Dry molasses	1.50
Dicalcium phosphate	1.25
Ground limestone	0.50
Salt	0.50
Premixes (vitamin and mineral)	0.25

(a) NIH, 1978; NCI, 1976
(b) Ingredients ground to pass through a U.S. Standard Screen No. 16 before being mixed

TABLE H2. VITAMINS AND MINERALS IN NIH 07 RAT AND MOUSE RATION (a)

	Amount	Source
Vitamin		
A	5,500,000 IU	Stabilized vitamin A palmitate or acetate
D_3	4,600,000 IU	D-activated animal sterol
K_3	2.8 g	Menadione activity
d-α-Tocopheryl acetate	20,000 IU	
Choline	560.0 g	Choline chloride
Folic acid	2.2 g	
Niacin	30.0 g	
d-Pantothenic acid	18.0 g	*d*-Calcium pantothenate
Riboflavin	3.4 g	
Thiamine	10.0 g	Thiamine mononitrate
B_{12}	4,000 µg	
Pyridoxine	1.7 g	Pyridoxine hydrochloride
Biotin	140.0 mg	*d*-Biotin
Mineral		
Iron	120.0 g	Iron sulfate
Manganese	80.0 g	Manganous oxide
Zinc	16.0 g	Zinc oxide
Copper	4.0 g	Copper sulfate
Iodine	1.4 g	Calcium iodate
Cobalt	0.4 g	Cobalt carbonate

(a) Per ton (2,000 lb) of finished product

TABLE H3. NUTRIENT COMPOSITION OF NIH 07 RAT AND MOUSE RATION

Nutrient	Mean ± Standard Deviation	Range	No. of Samples
Crude protein (percent by weight)	24.19 ± 1.07	22.4-26.3	25
Crude fat (percent by weight)	5.02 ± 0.47	4.2-6.0	25
Crude fiber (percent by weight)	3.37 ± 0.37	2.4-4.2	25
Ash (percent by weight)	6.54 ± 0.26	5.97-7.03	25
Essential Amino Acid (percent of total diet)			
Arginine	1.323 ± 0.830	1.21-1.39	4
Cystine	0.310 ± 0.099	0.218-0.400	4
Glycine	1.155 ± 0.069	1.06-1.21	4
Histidine	0.572 ± 0.030	0.530-0.603	4
Isoleucine	0.910 ± 0.033	0.881-0.944	4
Leucine	1.949 ± 0.065	1.85-1.99	4
Lysine	1.275 ± 0.076	1.20-1.37	4
Methionine	0.422 ± 0.187	0.306-0.699	4
Phenylalanine	0.909 ± 0.167	0.665-1.04	4
Threonine	0.844 ± 0.029	0.824-0.886	4
Tryptophan	0.187	0.171-0.211	3
Tyrosine	0.631 ± 0.094	0.566-0.769	4
Valine	1.11 ± 0.050	1.05-1.17	4
Essential Fatty Acid (percent of total diet)			
Linoleic	2.44	2.37-2.52	3
Linolenic	0.274	0.256-0.308	3
Arachidonic	0.008		1
Vitamin			
Vitamin A (IU/kg)	11,936 ± 2,547	8,900-22,000	25
Vitamin D (IU/kg)	4,650	3,000-6,300	2
α-Tocopherol (ppm)	41.53 ± 7.32	31.1-48.9	4
Thiamine (ppm) (a)	18.7 ± 3.20	14.0-26.0	24
Riboflavin (ppm)	7.5 ± 0.96	6.1-8.2	4
Niacin (ppm)	85.0 ± 14.2	65.0-97.0	4
Pantothenic acid (ppm)	29.3 ± 4.6	23.0-34.0	4
Pyridoxine (ppm)	7.6 ± 1.5	5.6-8.8	4
Folic acid (ppm)	2.8 ± 0.88	1.8-3.7	4
Biotin (ppm)	0.27 ± 0.05	0.21-0.32	4
Vitamin B_{12} (ppb)	21.0 ± 11.9	11.0-38.0	4
Choline (ppm)	3,302.0 ± 120.0	3,200.0-3,430	4
Mineral			
Calcium (percent)	1.22 ± 0.10	1.10-1.45	25
Phosphorus (percent)	0.96 ± 0.05	0.84-1.10	25
Potassium (percent)	0.862 ± 0.100	0.772-0.974	3
Chloride (percent)	0.546 ± 0.100	0.442-0.635	4
Sodium (percent)	0.311 ± 0.038	0.258-0.350	4
Magnesium (percent)	0.169 ± 0.133	0.151-0.181	4
Sulfur (percent)	0.316 ± 0.070	0.270-0.420	4
Iron (ppm)	447.0 ± 57.3	409.0-523.0	4
Manganese (ppm)	90.6 ± 8.20	81.7-95.5	4
Zinc (ppm)	53.6 ± 5.27	46.1-58.6	4
Copper (ppm)	10.77 ± 3.19	8.09-15.39	4
Iodine (ppm)	2.95 ± 1.05	1.52-3.82	4
Chromium (ppm)	1.81 ± 0.28	1.44-2.09	4
Cobalt (ppm)	0.68 ± 0.14	0.49-0.80	4

(a) One batch of feed (7/22/81) not analyzed for thiamine

TABLE H4. CONTAMINANT LEVELS IN NIH 07 RAT AND MOUSE RATION

Contaminant	Mean ± Standard Deviation	Range	No. of Samples
Arsenic (ppm)	0.65 ± 0.11	0.21-0.85	25
Cadmium (ppm) (a)	<0.1		25
Lead (ppm)	0.93 ± 0.78	0.27-2.93	25
Mercury (ppm) (a)	<0.05		25
Selenium (ppm)	0.28 ± 0.06	0.16-0.40	25
Aflatoxins (ppb) (a,b)	<10	<5.0-10.0	25
Nitrate nitrogen (ppm) (c)	9.85 ± 4.55	0.6-19.0	25
Nitrite nitrogen (ppm) (c)	1.92 ± 1.28	0.4-5.3	25
BHA (ppm) (d)	5.67 ± 5.07	1.5-20.0	25
BHT (ppm) (d)	3.35 ± 2.55	<1.0-13.0	25
Aerobic plate count (CFU/g) (e)	121,420 ± 94,844	7,000-420,000	25
Coliform (MPN/g) (f)	965 ± 991	<3-2,600	25
E. coli (MPN/g) (g)	6.76 ± 7.06	<3-23	24
E. coli (MPN/g) (h)	12.64 ± 29.46	<3-150	25
Total nitrosamines (ppb) (i,j)	4.40 ± 3.16	<1.2-12.9	24
Total nitrosamines (ppb) (i,k)	8.29 ± 19.41	1.2-100.3	25
N-Nitrosodimethylamine (ppb) (i,l)	3.05 ± 3.02	0.6-12.0	24
N-Nitrosodimethylamine (ppb) (i,m)	6.89 ± 19.42	0.6-99.0	25
N-Nitrosopyrrolidine (ppb)	1.20 ± 0.62	<0.3-2.4	25
Pesticide (ppm)			
α-BHC (a,n)	<0.01		25
β-BHC (a)	<0.02		25
γ-BHC-Lindane (a)	<0.01		25
δ-BHC (a)	<0.01		25
Heptachlor (a)	<0.01		25
Aldrin (a)	<0.01		25
Heptachlor epoxide (a)	<0.01		25
DDE (a)	<0.01	0.05 (7/14/81)	25
DDD (a)	<0.01		25
DDT (a)	<0.01		25
HCB (a)	<0.01		25
Mirex (a)	<0.01		25
Methoxychlor (p)	<0.05	0.13 (8/25/81); 0.6 (6/29/82)	16
Dieldrin (a)	<0.01		25
Endrin (a)	<0.01		25
Telodrin (a)	<0.01		25
Chlordane (a)	<0.05		25
Toxaphene (a)	<0.1		25
Estimated PCBs (a)	<0.2		25
Ronnel (a)	<0.01		25
Ethion (a)	<0.02		25
Trithion (a)	<0.05		25
Diazinon (a)	<0.1		25
Methyl parathion (a)	<0.02		25
Ethyl parathion (a)	<0.02		25
Malathion (q)	0.08 ± 0.05	<0.05-0.35	25
Endosulfan I (a)	<0.01		25
Endosulfan II (a)	<0.01		25
Endosulfan sulfate (a)	<0.03		25

TABLE H4. CONTAMINANT LEVELS IN NIH 07 RAT AND MOUSE RATION (Continued)

(a) All values were less than the detection limit, given in the table as the mean.
(b) The detection limit was reduced from 10 ppb to 5 ppb after 7/81.
(c) Source of contamination: Alfalfa, grains, and fish meal
(d) Source of contamination: Soy oil and fish meal
(e) CFU = colony-forming unit
(f) MPN = most probable number
(g) Mean, standard deviation, and range exclude one high value of 180 for the batch produced on 8/26/82.
(h) Mean, standard deviation, and range include the high value given in footnote (g).
(i) All values were corrected for percent recovery.
(j) Mean, standard deviation, and range exclude one value of 100.2 obtained for the batch produced on 4/27/81
(k) Mean, standard deviation, and range include the high value given in footnote (j).
(l) Mean, standard deviation, and range exclude one value of 90.0 obtained for the batch produced on 4/27/81
(m) Mean, standard deviation and range include the high value given in footnote (l).
(n) BHC = hexachlorocyclohexane or benzene hexachloride
(o) One observation was above the detection limit. The value and the date it was obtained are listed under the range.
(p) Two observations were above the detection limit. The values and the dates they were obtained are given under the range.
(q) Ten batches contained more than 0.05 ppm.

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APPENDIX I

DATA AUDIT SUMMARY

APPENDIX I. DATA AUDIT SUMMARY

The experimental data, records, and pathology materials at the NTP Archives for the 2-year toxicology and carcinogenesis studies of *N*-phenyl-2-naphthylamine in rats and mice were audited for accuracy, consistency, completeness, and compliance with Good Laboratory Practice regulations of the Food and Drug Administration (implemented by the NTP beginning on October 1, 1981). The laboratory experiments were conducted for the NTP by Battelle Columbus Laboratories, Columbus, Ohio, under a subcontract with Tracor Jitco, Inc., until October 1, 1982, and then under contract with the NIEHS. Exposure to the chemical in feed began on April 20, 1981, for rats and on May 11, 1981, for mice. The retrospective audit was conducted for the NTP in July 1986 by Argus Research Laboratories (Paul A. Wennerberg, D.V.M., Principal Investigator). The other individuals involved with the audit are listed in the full report of the audit which is on file at the NIEHS. The audit included a review of:

(1) All inlife records concerning animal receipt, quarantine, randomization, and disposition prior to study start.
(2) Clinical observations recorded during the last 6 months of life and all body weights for a random 10% sample of the study animals.
(3) All inlife records concerning environmental conditions, palpable masses, mortality, animal identification, and correlation of final inlife observation of masses, dates of death, and disposition with necropsy records.
(4) All chemistry records, including chromatograms, Midwest Research Institute reports and raw data, receipt reports, chemical use and dose preparation records, analytical records, and correspondence.
(5) All postmortem records for individual animals concerning identification, disposition codes, condition codes, and correlation between gross observations and microscopic diagnoses.
(6) Inventory and labeling for all wet tissue bags.
(7) Wet tissues from a random 20% sample of the study animals and from animals that had a gross observation without a corresponding microscopic diagnosis to verify animal identification and to examine for untrimmed lesions.
(8) Slides and blocks of tissues from all control and high dose animals to examine for proper match and inventory.
(9) The data pertaining to the 2-year studies of *N*-phenyl-2-naphthylamine in the Staff Review Draft of the NTP Technical Report.

The audit showed that the study records were complete. The daily observation records included several notations of wet cages and what was described as possible "dehydration" of animals, suggesting that the automatic watering system occasionally malfunctioned. The audit found that masses observed on animals during the last 2 months of life correlated with postmortem records; postmortem notations were not found for masses noted in only 10 rats (across all study groups) and one mouse.

The audit showed that the identities for 68/74 rats and 70/92 mice were correctly determined by examination of residual wet tissues. For those animals that could not be unequivocally identified, the identification marks were found to be either readable as another number, mutilated, or not all present. By reviewing the wet tissues of additional animals and by comparing the pattern of lesions removed from the wet tissues of individual animals that were not fully identified by markings with the description of lesions given on their necropsy record forms, it was possible to show that the integrity of animal identity had been maintained. Examination of about 7,000 individual wet tissues from 166 animals revealed only two untrimmed potential lesions (nontarget organs). There were three gross observations in rats (nontarget organs) which had no corresponding microscopic diagnosis.

All the findings from the retrospective data audit were reviewed and assessed by NTP staff. In conclusion, the study documents and specimens at the NTP Archives support the data and results presented in this NTP Technical Report.

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NATIONAL TOXICOLOGY PROGRAM TECHNICAL REPORTS
PUBLISHED AS OF JANUARY 1988

TR No.	CHEMICAL
201	2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (Dermal)
206	Dibromochloropropane
207	Cytembena
208	FD & C Yellow No. 6
209	2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (Gavage)
210	1,2-Dibromoethane (Inhalation)
211	C.I. Acid Orange 10
212	Di(2-ethylhexyl)adipate
213	Butylbenzyl Phthalate
214	Caprolactam
215	Bisphenol A
216	11-Aminoundecanoic Acid
217	Di(2-ethylhexyl)phthalate
219	2,6-Dichloro-*p*-phenylenediamine
220	C.I. Acid Red 14
221	Locust Bean Gum
222	C.I. Disperse Yellow 3
223	Eugenol
224	Tara Gum
225	D & C Red No. 9
226	C.I. Solvent Yellow 14
227	Gum Arabic
228	Vinylidene Chloride
229	Guar Gum
230	Agar
231	Stannous Chloride
232	Pentachloroethane
233	2-Biphenylamine Hydrochloride
234	Allyl Isothiocyanate
235	Zearalenone
236	D-Mannitol
237	1,1,1,2-Tetrachloroethane
238	Ziram
239	Bis(2-chloro-1-methylethyl)ether
240	Propyl Gallate
242	Diallyl Phthalate (Mice)
244	Polybrominated Biphenyl Mixture
245	Melamine
247	L-Ascorbic Acid
248	4,4'-Methylenedianiline Dihydrochloride
249	Amosite Asbestos
250	Benzyl Acetate
251	Toluene Diisocyanate
252	Geranyl Acetate
253	Allyl Isovalerate
255	1,2-Dichlorobenzene
257	Diglycidyl Resorcinol Ether
259	Ethyl Acrylate
261	Chlorobenzene
263	1,2-Dichloropropane
267	Propylene Oxide
269	Telone II®
271	HC Blue No. 1
272	Propylene
274	Tris(2-ethylhexyl)phosphate
275	2-Chloroethanol
276	8-Hydroxyquinoline
281	H.C. Red No. 3
282	Chlorodibromomethane
284	Diallylphthalate (Rats)
285	C.I. Basic Red 9 Monohydrochloride
287	Dimethyl Hydrogen Phosphite
288	1,3-Butadiene
289	Benzene
291	Isophorone
293	HC Blue No. 2
294	Chlorinated Trisodium Phosphate
295	Chrysotile Asbestos (Rats)
296	Tetrakis(hydroxymethyl)phosphonium Sulfate and Tetrakis(hydroxymethyl)phosphonium Chloride
298	Dimethyl Morpholinophosphoramidate
299	C.I. Disperse Blue 1
300	3-Chloro-2-methylpropene
301	o-Phenylphenol
303	4-Vinylcyclohexene
304	Chlorendic Acid
305	Chlorinated Paraffins (C_{23}, 43% chlorine)
306	Dichloromethane
307	Ephedrine Sulfate
308	Chlorinated Paraffins (C_{12}, 60% chlorine)
309	Decabromodiphenyl Oxide
310	Marine Diesel Fuel and JP-5 Navy Fuel
311	Tetrachloroethylene (Inhalation)
312	n-Butyl Chloride
314	Methyl Methacrylate
315	Oxytetracycline Hydrochloride
316	1-Chloro-2-methylpropene
317	Chlorpheniramine Maleate
318	Ampicillin Trihydrate
319	1,4-Dichlorobenzene
321	Bromodichloromethane
322	Phenylephrine Hydrochloride
323	Dimethyl Methylphosphonate
324	Boric Acid
325	Pentachloronitrobenzene
326	Ethylene Oxide
327	Xylenes (Mixed)
329	Methyl Carbamate

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